The Effect of a Mediterranean Diet versus Low-fat Diet on Inflammation in patients with Non-Alcoholic Fatty Liver Disease

Anjana Joy Reddy

BSc, HBHN (Hons)

Doctor of Philosophy

Thesis submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Dietetics, Nutrition and Sport
School of Allied Health, Human Services and Sport
College of Science, Health and Engineering
La Trobe University
Victoria, Australia

May 2021

Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver condition globally, affecting up to 30% of adults in Western countries. Chronic low-grade inflammation is a key feature in the pathogenesis of NAFLD and progression to steatohepatitis and cirrhosis. In the absence of effective pharmacotherapy, diet and weight loss are the main management strategies, however weight loss is often unsustainable. The Mediterranean diet (MedDiet), recognised for its anti-inflammatory properties and shown to improve cardiovascular health outcomes, is recommended in the management of NAFLD. Limited trials have assessed the effect of a MedDiet on inflammation in patients with NAFLD.

The primary aim of this thesis was to test the effect of a 12-week ad libitum MedDiet and LFD intervention on markers of inflammation. Secondary aims were to assess effects on liver outcomes, blood biomarkers, anthropometry, body composition, dietary inflammatory index (DII) and the prevalence of gene variants influencing levels of inflammatory markers in a multi-ethnic NAFLD cohort residing in Australia.

Forty-two participants (60% female, 52.3 ± 12.6 years, BMI 32.2 ± 6.2kg/m²; 43% type 2 diabetes mellitus) were recruited and randomised to the MedDiet (n=19) or LFD (n=23). The MedDiet group significantly improved adiponectin levels and significantly reduced visceral fat, in the absence of weight loss. No change in inflammatory markers was observed in the LFD group, however significant reduction in insulin resistance, liver enzymes and visceral fat occurred. Diet quality and adherence to the MedDiet improved in the entire cohort regardless of randomisation, and MedDiet adherence was associated with an improved DII score at 12-weeks. Significant inflammatory gene-nutrient interactions were not observed in this group. This study suggests that the MedDiet may improve inflammation and adiposity in patients with NAFLD, while an improvement in overall diet quality, regardless of dietary intervention, may be superior for secondary outcomes. Larger sample sizes are required to further explore these findings.

Statement of Authorship

"This thesis includes work by the author that has been published or accepted for publication as described in the text. Except where reference is made in the text of the thesis, this thesis contains no other material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgment in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution. All research procedures reported in the thesis were approved by the relevant Ethics Committee".

Anjana Joy Reddy

Date: 14/05/2021

Acknowledgements

Funding Support

List of Publications and Conference Abstracts

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

Acknowledgements

Dr Audrey Tierney

To my primary supervisor and first true mentor, Audrey. You have impacted my career in research and academia in the most profound way and for this I will forever be grateful. Thank you for your constant support and guidance over the years. I'm often in awe of the ease with which you conduct yourself through your teaching and research activities. I appreciate your kindness, generosity, down-to-earth nature and approachability, traits I feel I've developed as an academic, researcher and individual from working closely with you. Thank you.

Dr Elena George

To my co-supervisor, colleague, friend, and mentor – El. Thank you for taking me under your wing and introducing me to all-things MEDINA! You are a wealth of knowledge and I don't think that there was one meeting, phone-call or coffee catch-up where I didn't learn something new from you. I couldn't have asked for a more dynamic team and support network throughout my PhD candidature. Thank you for the moral support in tough and testing times, and for making sure to celebrate the small victories with me along the way.

Hepatologists and collaborators on the MEDINA trial; *Professor Stuart Roberts*, *Associate Professor Amanda Nicoll*, *Dr Marno Ryan, Dr Siddharth Sood*, *Dr Chee Kai Chan, Dr Paul Della-Gatta, Dr Shaun Mason, and all of the Alfred Health, Eastern Health and Melbourne Health staff.* Thank you for your involvement in planning and coordinating this project, guiding me through recruitment processes, data collection and analyses.

MEDINA Study Participants, without you all this work would not be possible. Thank you for your time and effort, it was lovely getting to know all of you and to grow your understanding of diet and disease.

PhD colleagues

To my amazing PhD colleagues both past and present; Elena, Gina, Sarah, Hannah, Oana, Brooke, Michael, Katerina, Elizabeth and Lauren, what would I have done without you all! Thanks for the (oh-so-many) conversations, coffee runs, lunch dates and laughs. It takes a village and a village we were!

La Trobe Nutrition and Dietetics Team

To the LTU Nutrition team both past and present, from those who have taught me to those I have taught with – thank you all. Thank you for your shoulders to lean on and those same shoulders to bolster me up and help me grow. I am so grateful.

I would like to acknowledge Dr Don Vicendese for providing statistical advice relating to each chapter of this doctoral thesis. A special mention to Dr Quynh Dinh and team, although we encountered a problematic analysis which has been put on hold for a later date, I appreciate all of your hard work, persistence, and time.

Family and Friends

To my girls, Mirella, Maddison, Chanel and Zoe, I've lost count of how many pep-talks (and wines) we have had in relation to my work. Thank you for listening, thank you for your support.

To my little loves, Priya and Kajal Reddy. You are the toughest girls I know with the gentlest hearts. Thank you for bringing light, laughter, and silliness into my life. Thank you for the endless conversations about changing the world and re-instilling kindness, compassion, and empathy in me when I'm feeling overwhelmed.

To my never sympathetic but always supportive brother, Adriel Reddy. Thanks for the tough love and perspective. I know I can count on you through the highs and the lows.

Finally, to the two people without whom I wouldn't be the woman that I am today, Mum and Dad. Thank you for all of the sacrifices you have made to get me to where I am today. Thank you for bringing me up in world where nothing was ever out of my reach, for always gently nudging me in the right direction and never pushing me, for your patience, unconditional love and understanding. Your strength, resilience and love for life is truly inspiring. You are what drives me to always do better. Thank you for trusting me to find my own way and believing in my journey.

This work is for you. With love, from your daughter, Dr Anj Reddy.

Funding Support

This thesis was embedded within the MEDINA trial which was funded by an Understanding Disease Research Focus Area Internal La Trobe University grant. The investigators; *Tierney AC, Roberts SK, Itsiopoulos C, Jois M, Salim A, Ryan M, Nicoll AJ, Roche H, George ES.*

The analysis of inflammatory cytokine and adipokine markers within the MEDINA trial was funded by an Australasian Society of Parenteral and Enteral Nutrition small project grant. The investigators; *George ES, Tierney AC, Roberts SK, Nicoll AJ, Itsiopoulos C, Reddy AR.*

Anjana J Reddy was supported by an Australian Government Research Training Program Scholarship.

Thank you to the following companies for the generous donations for the food hampers provided to participants on the trial:

- *Boundary Bend:* Cobram Estate Extra Virgin Olive Oil;
- *Heinz:* canned tuna and baked beans;
- *Simplot:* canned tuna and canned lentils and legumes;
- *Australian Almond Board:* almonds;
- *Jalna*: yogurt;
- *Carman's:* muesli bars.

Individuals who made donations to participants on the trial;

- Professor Catherine Itsiopoulos for donating the Mediterranean Diet Cookbooks.

List of Publications and Conference Abstracts

Publications

Reddy, A. J., George, E. S., Roberts, S. K., & Tierney, A. C. (2019). Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with non-alcoholic fatty liver disease: a systematic literature review. *Nutrition Reviews*, *77*(11), 765-786. https://doi:10.1093/nutrit/nuz029

George, E. S., Marshall, S., Mayr, H. L., Trakman, G. L., Tatucu-Babet, O. A., Lassemillante, A. C. M., Bramley, A., **Reddy, A. J**., Forsyth, A., Tierney, A. C & Thomas, C. J. (2019). The effect of high-polyphenol extra virgin olive oil on cardiovascular risk factors: A systematic review and meta-analysis. *Critical reviews in food science and nutrition, 59*(17), 2772-2795. https://doi.org/10.1080/10408398.2018.1470491

George, E. S., Roberts, S. K., Nicoll, A. J., **Reddy, A**. **J**., Paris, T., Itsiopoulos, C., & Tierney, A. C. (2018). Non-alcoholic fatty liver disease patients attending two metropolitan hospitals in Melbourne, Australia: high risk status and low prevalence. *Internal medicine journal*, *48*(11), 1369-1376. https://doi.org/10.1111/imj.13973

Thesis Publications (preparing for submission)

Reddy, A. J., Tierney, A. C., Roberts, S. K., Nicoll, A. J., Sood, S., Della Gatta, P., Mason, S., & George, E. S. The effect of a 12-week Mediterranean Diet versus Low-Fat Diet on Inflammation in patients with Non-Alcoholic Fatty Liver Disease: results of the MEDINA study (Original Article). Target journal: *Nutrients.* Impact Factor 4.546

Reddy, A. J., George, E. S., Della Gatta, P., Mason, S., Hebert, J., Shivappa, N., & Tierney, A. C. Changes in dietary inflammatory index (DII), adiposity and markers of inflammation and fatty liver disease in patients with Non-Alcoholic Fatty Liver Disease, following an improvement in diet quality (Original Article). Target journal: *Nutrition Research.* Impact Factor 2.767

Reddy, A. J., George, E. S., Chan, C. K., & Tierney, A. C. Single nucleotide polymorphisms (SNPs) related to inflammation and their association with circulating inflammatory markers and

features of NAFLD before and after a dietary intervention to improve diet quality: the MEDINA study (Original Article). Target Journal: *Genes & Nutrition.* 2-year Impact Factor 4.258

Reddy, A. J., George, E. S., Nicoll, A. J., Sood, S., Ryan, M., Roberts, S. K., & Tierney, A. C. (2018). Prevalence and associations of Interleukin Variants in patients with Non-Alcoholic Fatty Liver Disease. *Journal of Gastroenterology and Hepatology,* Vol. 33, pp. 69-70

Reddy, A. J., George, E. S., Nicoll, A. J., Sood, S., Ryan, M., Roberts, S. K., & Tierney, A. C. (2017) Prevalence and associations of inflammatory genetic polymorphisms in a Non-Alcoholic Fatty Liver Disease Cohort. *Journal of Gastroenterology and Hepatology, 32*(Suppl. 2): 87–115. https://doi.org/10.1111/jgh.13893. Conference: Gastroenterology Society of Australia (GESA) - Australian Gastroenterology Week 2017

Reddy A. J., George, E. S., Ryan, M. C., Sood, S., Nicoll, A., Roberts, S. K., & Tierney, A. C. (2017). Interleukin-6 -174G/C Polymorphism in Non-Alcoholic Fatty Liver Disease Prevalence and Metabolic Characteristics. *Scripta Scientifica Pharmaceutica, 4*(1). http://dx.doi.org/10.14748/ssp.v4i1.4008. Conference: the Annual Nutrigenomics Organisation (NuGO) Conference Week 2017 in Varna, Bulgaria.

George, E. S., **Reddy, A. J**., Nicoll, A. J., Sood, S., Ryan, M., Johnson, N. A., ... & Tierney, A. C. (2018). The effect of a Mediterranean diet and low-fat diet on intrahepatic fat, liver stiffness and insulin resistance in patients with non-alcoholic fatty liver disease: Preliminary findings from the MEDINA Trial. *Journal of Gastroenterology and Hepatology,* Vol. 33, pp. 141-142.

George, E. S., Forsyth, A., **Reddy, A. J**., Itsiopoulos, C., & Tierney, A. (2018) A Mediterranean and Low Fat dietary intervention in Non-Alcoholic Fatty Liver Disease patients; Exploring participant experience and perceptions about dietary change. *Nutrition and Dietetics*, *75* (Suppl. 1): 28-28. Conference: Dietitians Association of Australia 35th Annual Conference.

Tierney, A. C., George, E., **Reddy, A**. **J**., Ryan, M., Sood, S., Nicoll, A., & Roberts, S. (2017). SUN-LB311: FTO RS9939609 Polymorphism in Non-Alcoholic Fatty Liver Disease – Frequency and Characteristics of Risk Genotypes. *Clinical Nutrition*, *36*, S169. https://doi.org/10.1016/S0261-5614(17)30652-0

George, E. S., Roberts, S. K., Nicoll, A. J., **Reddy, A**., Paris, T., Itsiopoulos, C., & Tierney, A. C. (2017). High-risk status and low prevalence of patients with non-alcoholic fatty liver disease attending metropolitan liver clinics in Australia. *Journal of Gastroenterology and Hepatology,* Vol. 32, pp. 92-93.

Other Conference Presentations

Reddy, A. J., George, E. S., Roberts, S. K., & Tierney, A. C. (2019). Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with nonalcoholic fatty liver disease: a systematic literature review. *Dietitians Association of Australia 36th National Conference, More Than Meets the Eye, 12-14 August 2019, Gold Coast, Australia.*

Invited Presentations

Baw Baw Sustainability Network (BBSN), 2019, 'The Mediterranean Diet and You', invited speaker

Table of Contents

1 Introduction 32

1.1 Non-Alcoholic Fatty Liver Disease (NAFLD) 32

1.1.1 Definition 32

1.1.2 Overview of the Role of the Liver: Carbohydrate and Lipid Metabolism 33

1.1.3 Epidemiology 35

1.1.4 Risk Factors and Clinical Features of NAFLD 38

1.1.5 Common Metabolic Disorders related to NAFLD 41

1.1.1 Clinical Presentation 44

1.1.2 From NAFLD to MAFLD 44

1.1.3 Pathogenesis of NAFLD 47

1.1.4 Inflammation and NAFLD 49

1.1.5 Genes influencing inflammation in NAFLD 54

1.1.6 Assessment and Diagnosis of NAFLD 56

1.1.7 Management Strategies 61

1.2 Habitual Dietary Intakes in NAFLD 64

1.2.1 Dietary Approaches to the Management of NAFLD 65

1.2.2 The Low-Fat Diet 67

1.2.3 The Mediterranean Diet 67

1.3 The impact of diet on inflammation in NAFLD 71

1.3.1 Effect of Dietary Intervention on Inflammatory Markers in patients with NAFLD: A Systematic Review 72

1.3.2 Update to the Systematic Literature Review: Effect of Dietary Intervention on Inflammatory Makers in patients with NAFLD 73

1.3.3 Mediterranean Diet and Inflammatory Status in NAFLD 80

1.3.1 Evidence for Gene-Diet Interaction in NAFLD 82

1.4 Dietary Inflammatory Index 84

1.4.1 Development and Validation of the DII 85
1.4.2 Application of DII in NAFLD 86
1.5 Summary of Chapter 1 87
1.6 Thesis Preface 88
1.7 Thesis Aims and Research Questions 88
1.8 Thesis Structure 89
2 Methods 92
2.1 Chapter Overview 92
2.2 MEDINA Study Design 92
2.3 MEDINA Overall Study Aims 92
2.4 Participant Eligibility and Screening 93
2.5 Recruitment and Randomisation 94
2.6 Timeline of Appointments 95
2.7 Dietary Intervention Arms 95
2.7.1 Mediterranean Diet 96
2.7.2 Low-Fat Diet 98
2.8 Data Collection and Outcome Measures 99
2.8.1 Demographic data 99
2.8.2 Primary Outcomes; Inflammatory Markers 99
2.8.3 Secondary Outcomes 100
2.8.4 Questionnaires 106
2.8.5 Dietary Inflammatory Index 113
2.9 Genotyping 114
2.10 Ethics Approval 114
2.11 Sample Size 114
2.12 Data Analysis 115
2.13 Investigator Involvement in Research Tasks 115
3 Sex and metabolic differences in patients with NAFLD: 118
The MEDINA Study Cohort at Baseline 118
3.1 Abstract 118
3.2 Introduction 119

3.3 Methods....122
3.3.1 Study Design....122
3.3.2 Eligibility criteria....122
3.3.3 Screening and Recruitment....122
3.3.4 Data collection and outcome measures assessed....123
3.3.5 Metabolic Syndrome Diagnosis....125
3.3.6 Statistical Analysis....125
3.4 Results....127
3.4.1 Demographics of the NAFLD cohort....127
3.4.2 Anthropometry and body composition....129
3.4.3 Biochemical profile....131
3.4.4 Inflammatory Profile....133
3.4.5 Liver outcomes....133
3.4.6 Dietary intake....135
3.4.7 Food Group Intake....139
3.4.8 Associations of Anthropometric Measurements and Biochemical Markers with Inflammatory cytokines and adipokines in a NAFLD cohort....142
3.4.9 Associations between Inflammatory Markers at Baseline....143
3.4.10 Associations of Nutrients and Food Group Intake with Markers of Inflammation in a NAFLD cohort....144
3.4.11 Summary of Significant (Positive and Negative) Correlations....146
3.4.12 Multiple Regression Analysis....147
3.5 Discussion....153
3.5.1 Conclusion....159
4 The effect of a Mediterranean Diet versus Low-Fat Diet on Inflammation in patients with Non-Alcoholic Fatty Liver Disease: the MEDINA Randomised Controlled Trial....161
4.1 Abstract....161

4.2 Introduction 162
4.3 Section One: The effect of a Mediterranean Diet versus a Low-Fat Diet on inflammation in NAFLD 166
4.3.1 Aims 166
4.3.2 Hypotheses: 167
4.4 Section Two – Subgroup Analysis: 167
Assessing the impact of a dietary intervention and overall improved diet quality in patients diagnosed with NAFLD and T2DM (n=18). 167
4.4.1 Rationale: 167
4.4.2 Aims 167
4.4.3 Hypotheses: 167
4.5 Methods 168
4.5.1 Study Design 168
4.5.2 Participant Screening and Recruitment 168
4.5.3 Eligibility Criteria 168
4.5.4 Dietary Interventions 169
4.5.5 Demographic Data 170
4.5.6 Anthropometric, Haemodynamic and Body Composition Measures 170
4.5.7 Primary Outcome Measures – Inflammatory Markers 171
4.5.8 Secondary Outcome Measures 171
4.5.9 Dietary Assessment 172
4.5.10 PREDIMED Score 172
4.5.11 Statistical Analysis 173
4.5.12 Power Calculation 176
4.5.13 Ethics 177
4.6 Results 177
4.6.1 Population Characteristics 177
4.7 Section One – The effects of a dietary intervention in patients with NAFLD 178

4.7.1 Differences between the MedDiet and LFD intervention groups for Inflammatory Markers at 12-weeks – end intervention and absolute change data 178

4.7.2 Effects of the Dietary Intervention on Inflammatory Markers– within diet group effects 179

4.7.3 Differences between the MedDiet and LFD intervention groups for Liver Outcomes and Biochemistry at 12-weeks – end intervention and absolute change data 179

4.7.4 Effects of the Dietary Intervention on Liver Outcomes and Biochemistry 180

4.7.5 Effects of Dietary Intervention on Anthropometry, Body Composition and Haemodynamic measures 186

4.7.6 Effect of the LFD and MedDiet on Nutrient and Food Group Intake 191

4.7.7 Dietary intakes in the LFD and MedDiet study arms at end of intervention (12-weeks) compared to prescribed dietary interventions 204

4.7.8 Correlations 205

4.7.9 Diet Quality 211

4.7.10 Diet Quality in a NAFLD cohort – pooled analysis 215

4.7.11 Regression analysis: Components of a MedDiet pattern with inflammatory markers 220

4.7.12 Regression analysis: liver outcomes, biochemistry, anthropometry, and body composition with inflammatory markers 226

4.8 Section Two – Assessing the impact of a dietary intervention and overall improved diet quality in patients diagnosed with NAFLD and T2DM – Subgroup Analysis 233

4.8.1 The effect of a LFD vs a MedDiet within the NAFLD Type 2 Diabetes Cohort .. 233

4.8.2 Population Characteristics 233

4.8.3 Inflammatory Markers 233

4.8.4 IHL and LSM 234

4.8.5 Blood Biomarkers 234

4.8.6 Anthropometry and Body Composition 235

4.8.7 Adherence to a Mediterranean Diet 235

4.8.8 Analysis of Diabetes Status in the Pooled Patient Cohort, Irrespective of Diet Group Allocation 240

4.9 Discussion 245
4.10 Linking chapter 4 and 5 256

5 Dietary Inflammatory Index, Diet Quality and Markers of Inflammation in patients with Non-Alcoholic Fatty Liver Disease following a 12-week Dietary Intervention. 258

5.1 Abstract 258
5.2 Introduction 259
5.3 Methods 261
5.3.1 Study Design and Participants 261
5.3.2 Dietary Interventions 262
5.3.3 Primary Outcome Measures – Dietary Inflammatory Index (DII®) 263
5.3.4 Secondary Outcome Measures 264
5.3.5 Statistical Analysis 266
5.4 Results 267
5.4.1 DII score of the NAFLD cohort 267
5.4.2 DII and Dietary Intake in a NAFLD cohort 270
5.4.3 DII and Diet Quality (Adherence to the MedDiet) 272
5.4.4 Components of a MedDiet and DII 273
5.4.5 DII and inflammatory markers and clinical characteristics 277
5.5 Discussion 279
5.6 Linking chapter 5 and 6 287

6 Single Nucleotide Polymorphisms in Inflammatory Genes may modify disease-risk and interact with nutrients to modulate serum cytokine levels in patients with NAFLD 288

6.1 Abstract 288
6.2 Introduction 289
6.3 Methods 293
6.3.1 Study Design and Participants 293
6.3.2 Diagnosis of Type 2 Diabetes Mellitus and the Metabolic Syndrome 294

6.3.3 Dietary Intervention and Timepoints 294
6.3.4 Genotyping 295
6.3.5 Study Outcomes 296
6.3.6 Statistical analysis 297
6.4 Results 301
6.4.1 Genotype and allele frequency of SNPs CRP +1846C/T, IL-6 -174G/C, TNF-α -308A/G and adiponectin +276G/T in a NAFLD cohort 301
6.4.2 Baseline characteristics of the study population according to genotype of SNPs CRP +1846C/T, IL-6 -174G/C, TNF-α -308A/G and adiponectin +276G/T 302
6.4.3 Associations between gene variants with risk factors of the Metabolic Syndrome 303
6.4.4 Associations between gene variants with Type 2 Diabetes Mellitus 304
6.4.5 Differences in inflammatory, biochemistry and haemodynamic markers, liver outcomes, and anthropometric and body composition variables across the genotypes at baseline and post 12-week dietary intervention 305
6.4.6 The influence of inflammatory gene variants' on circulating inflammatory markers in a NAFLD cohort 311
6.4.7 Genotype Nutrient Interactions 315
6.4.8 Dietary Adherence: Genotype x MedDiet Interactions 319
6.5 Discussion 322
7 Discussion 328
7.1 Key Findings 328
7.2 Strengths and Limitations 336
7.3 Future Recommendations and Implications 339
7.4 Conclusion 341

List of Figures

Chapter 1

Figure 1.1 Model illustrating (A) normal response of the liver to a glucose load and (B) selective insulin resistance in liver with type 2 diabetes mellitus. *Adapted from Brown and Goldstein (2008).*

Figure 1.2 MAFLD: an 'old' new disease. *Adapted from Tilg and Effenberger (2020).*

Figure 1.3 The Multiple Hit Hypothesis for the development of NAFLD. *Adapted from Buzzetti et al. 2016.*

Figure 1.4 Secretion of inflammatory adipokines from enlarged adipose tissue in an obese state. *Adapted from Jung and Choi (2014).*

Figure 1.5 Proposed anti-inflammatory mechanisms of the Mediterranean diet and resultant benefit for NAFLD and other cardiometabolic diseases. *Adapted from Mirmiran et al. (2017) and Esposito et al. (2017)*

Figure 1.6 Nutrigenomics and Nutrigenetics are resulted from genes and nutrients interaction. *Adapted from Farhud, Yeganeh and Yeganeh (2010).*

Figure 1.8 A summary of the structure of this doctoral thesis and an outline of the key components of each research chapter

Chapter 2

Figure 2.1 The Mediterranean Diet hamper provided to participants in the MedDiet intervention group at baseline and mid-intervention timepoints

Figure 2.2. Example of the Frankfurt Plane for measurement of height using a stadiometer

Figure 2.3 Example of measurement protocol for waist circumference

Figure 2.4 Example of measurement location for neck circumference

Figure 2.5 Summary of participant face to face appointment at baseline, 6-weeks, and 12-weeks. Reprinted with permission from George, E. S. (2017). A Mediterranean diet for the management of non-alcoholic fatty liver disease (Doctoral thesis, La Trobe University, Melbourne, Australia). Retrieved from http://hdl handle.net/1959.9/564467

Chapter 4

Figure 4.1 Study patient flow chart for the MEDINA Study. Summary of participant recruitment and number of participants whose data was collected at each timepoints

Figure 4.2 PREDIMED Checklist Scores for adherence to a LFD and adherence to a MedDiet,
at baseline and end intervention time points

Figure 4.3 PREDIMED Scores for adherence to a Mediterranean Diet within the LFD and MedDiet groups at baseline and end-intervention

Figure 4.4 Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the LFD group at baseline and end-intervention

Figure 4.5 Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the MedDiet group at baseline and end-intervention

Figure 4.6 PREDIMED Scores of Mediterranean Diet adherence for the entire cohort of MEDINA participants at baseline and end-intervention

Figure 4.7 Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the overall NAFLD cohort at baseline and end-intervention

Chapter 5

Figure 5.1 Study flow diagram of the pooled cohort of MEDINA participants, distribution is categorised by anti-inflammatory (<0.00) versus pro-inflammatory (>0.00) DII score at study timepoints and comparison of diet study groups

Figure 5.2 The DII score of participants classified as adhering versus not adhering to individual components of a MedDiet, based on the PREDIMED checklist

Chapter 6

Figure 6.1 Frequency of genotypes of common single nucleotide polymorphisms (SNPs) CRP +1846C/T (rs1205), IL-6 -174G/C (rs1800795), TNF-α -308A/G (rs1800629) and adiponectin +276G/T (rs1501299)

Figure 6.2 Effect of the TNF-α -308A/G single nucleotide polymorphism and energy intake (kJ) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.3 Effect of the TNF-α -308A/G single nucleotide polymorphism and protein intake on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.4 Effect of the TNF-α -308A/G single nucleotide polymorphism and carbohydrate intake on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.5 Effect of the TNF-α -308A/G single nucleotide polymorphism and total sugar intake on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet interventions

Figure 6.6 Effect of the TNF-α -308A/G single nucleotide polymorphism and monounsaturated fatty acid intake on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.7 Effect of the TNF-α -308A/G single nucleotide polymorphism and fat intake on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.8 Effect of the CRP +1846C/T single nucleotide polymorphism and adherence to the Mediterranean Diet on change in high-sensitivity C-reactive protein (hs-CRP) concentration between baseline and end of diet intervention

Figure 6.9 Effect of the TNF-α -308A/G single nucleotide polymorphism and adherence to the Mediterranean Diet on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline and end of diet intervention

Figure 6.10 Effect of the IL-6 -174G/C single nucleotide polymorphism and adherence to the Mediterranean Diet on change in interleukin-6 (IL-6) concentration between baseline and end of diet intervention

Figure 6.11 Effect of the adiponectin +276G/T single nucleotide polymorphism and adherence to the Mediterranean Diet on change in adiponectin concentration between baseline and end of diet intervention

List of Tables

Chapter 1

Table 1.1 Characteristics of studies included for the updated systematic review investigating the effects of dietary intervention(s), with or without co-intervention, on inflammatory markers in adults with non-alcoholic fatty liver disease

Table 1.2 Dietary intervention protocol data extracted from each study included in the update to systematic review

Chapter 2

Table 2.1 The MEDINA Study Eligibility Criteria

Table 2.2 Nutrient composition of the Australian Mediterranean Diet. *Adapted from George et al. 2018.*[1]

Table 2.3 Summary of data collected at MEDINA Study appointments during the 12-week intervention

Table 2.4 Summary of the pathology markers measured with corresponding normal reference ranges

Table 2.5 Overview of research tasks related to this thesis and involvement of the Candidate and other investigators

Chapter 3

Table 3.1 Demographics of a NAFLD cohort (n=42)

Table 3.2 Baseline anthropometry and body composition characteristics of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.3 Baseline biochemistry characteristics of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.4 Baseline cytokine and adipokine profile of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.5 Baseline liver fat and stiffness measurements of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.6 Baseline dietary intake of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.7 Baseline food group intake of the NAFLD cohort, split by gender or presence of metabolic syndrome (MetS)

Table 3.8 Correlations between anthropometric and biochemical variables at baseline

Table 3.9 Correlations between inflammatory markers at baseline

Table 3.10 Correlations between Nutrient and Food Group Intake with Markers of Inflammation at baseline

Table 3.11 Multiple linear regression model for the outcome variable hs-CRP at baseline

Table 3.12 Multiple linear regression model for the outcome variable TNF-α at baseline

Table 3.13 Multiple linear regression model for the outcome variable IL-6 at baseline

Table 3.14 Multiple linear regression model for the outcome variable Adiponectin at baseline

Table 3.15 Multiple linear regression model for the outcome variable Resistin at baseline

Table 3.16 Multiple linear regression model for the outcome variable Leptin at baseline

Chapter 4

Table 4.1 Inflammatory cytokine and adipokine markers and liver outcomes at baseline and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Table 4.2 HOMA-IR and biochemical measures at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Table 4.3 Anthropometry, body composition and haemodynamic measures at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Table 4.4 Nutrient intake variables at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Table 4.5 Mean food group intake at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Table 4.6 Daily nutrient intake profile of the prescribed Mediterranean Diet and actual nutrient intake of participants in the MedDiet arm of the MEDINA Study at 12-weeks

Table 4.7 Partial correlations between inflammatory markers with liver, biochemical, anthropometric and body composition variables in each diet group at end intervention, values adjusted for mean weight-loss

Table 4.8 Partial correlations between inflammatory markers with nutrient and food group intake in each diet group at end intervention, values adjusted for mean weight-loss

Table 4.9 Mean values of inflammatory markers and key liver, metabolic and anthropometric measures for the entire MEDINA cohort at end-intervention, according to level of adherence to a MedDiet (PREDIMED scores)

Table 4.10 Partial correlations between inflammatory markers with nutrients and food groups in a Mediterranean-style dietary pattern (isolated dietary intake components of the PREDIMED checklist), with values adjusted for baseline inflammatory marker

Table 4.11 Multiple linear regression model for components of a MedDiet pattern with outcome variable hs-CRP at the end-intervention

Table 4.12 Multiple linear regression model for components of a MedDiet pattern with outcome variable IL-6 at end-intervention

Table 4.13 Multiple linear regression model for components of a MedDiet pattern with outcome variable Leptin at end-intervention

Table 4.14 Partial correlations between inflammatory markers, anthropometric and biochemical variables at end intervention, values adjusted for mean weight-loss

Table 4.15 Partial correlations between inflammatory markers, nutrient and dietary food group variables at end intervention, values adjusted for mean weight-loss

Table 4.16 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable hs-CRP at the end-intervention

Table 4.17 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable TNF-α at the end-intervention

Table 4.18 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable IL-6† at the end-intervention

Table 4.19 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Adiponectin† at the end-intervention

Table 4.20 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Leptin at the end-intervention

Table 4.21 Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Resistin at the end-intervention

Table 4.22 Mean values for inflammatory markers, liver outcomes and key biochemical, anthropometric and body composition variables at baseline and end-intervention timepoints, by dietary intervention arm (LFD vs. MedDiet) and diagnosis of T2DM

Table 4.23 Mean values for inflammatory markers, liver outcomes and key biochemical, anthropometric and body composition variables at baseline and end-intervention timepoints, categorised diagnosis of T2DM

Chapter 5

Table 5.1 Baseline to 12-week change in PREDIMED scores for MedDiet adherence and nutrient and food group intake across change in DII groups

Table 5.2 Partial correlations between change in DII and nutrient and food group (change) variables, values adjusted for baseline nutrient/food group variable

Table 5.3 Levels of dietary adherence (based on the PREDIMED score) at baseline and end intervention timepoints and associated mean DII score

Table 5.4 Mean change in DII of participants adhering and not adhering to individual components of the PREDIMED Checklist at 12-weeks

Table 5.5 Dietary Inflammatory Index scores of participants adhering versus not adhering to individual components of the PREDIMED Checklist baseline and 12-weeks

Table 5.6 Baseline to 12-week change in inflammatory markers, liver outcomes, biochemistry and clinical characteristics across change in DII groups

Table 5.7 Partial correlations between DII score at the end of intervention with inflammatory markers, liver outcomes, biochemistry and clinical characteristics at the end of intervention, values adjusted for change in energy intake (kJ)

Chapter 6

Table 6.1 Candidate SNPs and their descriptions

Table 6.2 Allele and genotype count and frequency for CRP +1846, IL-6 -174, TNF-α -308, and adiponectin +276 gene variants

Table 6.3 Baseline characteristics of the NAFLD cohort, split by candidate SNPs and genotypes

Table 6.4 Association of IL-6 -174 polymorphism with the likelihood of having the Metabolic Syndrome in patients with NAFLD

Table 6.5 Association of adiponectin +276 polymorphism with type 2 diabetes mellitus in patients with NAFLD

Table 6.6 Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to CRP +1846C/T genotype

Table 6.7 Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to IL-6 -174G/C genotype

Table 6.8 Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to TNF-α -308A/G genotype

Table 6.9 Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to adiponectin +276G/T genotype

Table 6.10 Multiple linear regression models for the outcome variables of inflammatory markers hs-CRP, IL-6, TNF-α and adiponectin

List of Appendices

Appendix 1 Published manuscript and supplementary materials published in association to the article

Appendix 2 Food parameters included in the dietary inflammatory index, inflammatory effect scores, and intake values from the global composite data set; Dietary Inflammatory Index Development Study, Columbia, SC, USA, 2011–2012

Appendix 3 Participant Information Consent Form (PICF) and Common Research Forms (CRF's)

- *3.1 PICF*
- *3.2 Screening Form*
- *3.3 Personal Information Form*
- *3.4 Anthropometry Form*
- *3.5 Dietary consultation form*

Appendix 4 Mediterranean Dietary Intervention Resources

Appendix 5 Alfred Pathology

Appendix 6 **Questionnaires**

- *6.1 3-day food diary*
- *6.2 PREDIMED Checklist for the Mediterranean Diet*
- *6.3 PREDIMED Checklist for the Low-Fat Diet*

Appendix 7 The serve sizes for the Xyris, Foodworks, Food Groups

Appendix 8 Ethics Approval Certificates

- *8.1 La Trobe University*
- *8.2 Alfred Health*
- *8.3 Eastern Health*
- *8.4 Melbourne Health*

Appendix 9 Power calculations for inflammatory markers (Chapter 4)

Appendix 10 Consort Checklist

Appendix 11 Supplementary Materials

Supplementary Table 1. Associations of Anthropometric Measurements and Biochemical Markers with Inflammatory cytokines and adipokines in a NAFLD cohort

Supplementary Table 2. Associations of Nutrients and Food Group Intake with Markers of Inflammation in a NAFLD cohort

Supplementary Table 3. Effects of Dietary Intervention on Anthropometry, Body Composition and Haemodynamic measures

Supplementary Table 4. Effects of Dietary Intervention on Nutrient and Food Group Intake

Supplementary Table 5. Correlation coefficients between inflammatory markers and other inflammatory markers at the end-intervention timepoint

Supplementary Table 6. Correlation coefficients for primary inflammatory outcomes and nutrient and dietary food group intake, at the end-intervention timepoint, non-significant variables

Supplementary Material 7. Point-biserial correlations between body mass index with IL-6 -174 genotypes at end intervention

Supplementary Table 8. Point-biserial correlations between variables at each timepoint with TNF-α -308 genotypes

List of Abbreviations

APD	Accredited Practicing Dietitian
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
BIA	Bioelectrical Impendence Analysis
BMI	Bod Mass Index
BP	Blood Pressure
CHD	Coronary Heart Disease
CHO	Carbohydrate
CRF	Common Research Forms
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DEXA	Dual Energy X-ray Absorptiometry
DII	Dietary Inflammatory Index
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acid
DNL	De Novo Lipogenesis
EPA	Eicosapentaenoic Acid
EVOO	Extra Virgin Olive Oil
FAs	Fatty Acids
Fox01	Forkhead box protein O1
GGT	Gamma-Glutamyl Transferase
G6P	Glucose 6-Phosphatase
GWAS	Genome-Wide Association Studies
HbA1c	glycosolated haemoglobin
HCC	Hepatocellular Carcinoma
HDL	High Density Lipoprotein
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
^{1}H-MRS	proton magnetic resonance spectroscopy
hs-CRP	high sensitive C-Reactive Protein
IHL	Intrahepatic Lipid

IL-6	Interleukin-6
IR	Insulin Resistance
LCN3s	Long Chain Omega 3 Fatty Acids
LDL	Low Density Lipoprotein
LFD	Low Fat Diet
LSM	Liver Stiffness Measure
MEDINA	Mediterranean Dietary Intervention for patients with Non-alcoholic fatty liver disease study
MedDiet	Mediterranean diet
MetS	the Metabolic Syndrome
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MUFA	Monounsaturated Fatty Acids
n-3	Omega-3 Fatty Acids
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NHMRC	National Health and Medical Research Council
PICF	Patient Information and Consent Form
PNPLA3	Patatin-like phospholipase domain-containing protein
PREDIMED	Prevención con Dieta Mediterránea
PUFA	Polyunsaturated Fatty Acids
RCT	Randomised Controlled Trial
SBP	Systolic Blood pressure
SFA	Saturated Fatty Acids
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
SREBP	Sterol-Regulatory-Element Binding Protein
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TE	Transient Elastography
TG	Triglycerides
TNF-α	Tumor Necrosis Factor-Alpha

VF	Visceral Fat
vLDL	very Low Density Lipoprotein
WHO	World health organisation

1 Introduction

1.1 Non-Alcoholic Fatty Liver Disease (NAFLD)

1.1.1 Definition

Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of hepatic triglycerides exceeding 5% of liver weight, in the absence of excess alcohol intake or additional aetiologies of liver disease including but not limited to; hepatitis B or hepatitis C, autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), genetic diseases (haemochromatosis, Wilsons disease, alpha-1 anti-trypsin (A1AT)) and drug-induced liver disease. While the preliminary form of NAFLD as simple steatosis may remain uncomplicated, 30-40% of people with steatosis will develop non-alcoholic steatohepatitis (NASH)[2] which encompasses hepatocellular damage and lobular necroinflammation, with or without fibrosis, and further cirrhosis.[3, 4] Further to this, the clinical implications of NAFLD involve its potential to progress to advanced liver disease, hepatocellular carcinoma (HCC) and liver failure.[5]

The first step in the development of NAFLD is represented by the accumulation of fat in the liver, or more specifically intrahepatic lipid (IHL) content above 5%.[6] Often the only laboratory abnormality found in patients with NAFLD is mild to moderately elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), or both.[5] While these liver enzymes are commonly used as an indication of abnormal liver function, the normal ratio of AST to ALT increases as fibrosis advances leading to a loss of diagnostic accuracy in patients with more advanced/cirrhotic NAFLD.[7] Serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are above the normal range in NAFLD, though the degree of elevation is less than those with alcoholic liver disease.[8] Hypoalbuminemia and hyperbilirubinemia may be found in patients with cirrhotic NAFLD, while approximately half of patients have elevated serum ferritin and 6-11% of patients have increased transferrin saturation.[5, 7] It is recognised that cryptogenic cirrhosis shares many of the same clinical features as NAFLD, and a high proportion of patients previously diagnosed with cryptogenic cirrhosis in fact had unrecognized NAFLD.[9] Liver enzymes are used as a non-invasive means of screening for patients with NAFLD, however they lack the specificity and sensitivity to diagnose NAFLD or NASH and stage of severity.[10]

Alcohol consumption history must be obtained accurately and comprehensively in order to ensure the correct diagnosis is made between NAFLD and alcoholic liver disease, as the difference cannot be distinguished through imaging and histological assessment.[11] The American Association for the Study of Liver Diseases has previously defined an acceptable alcohol limit as <20 g of ethanol /day for females and <30 g of ethanol /day for males.[12] Liver biopsies remain the gold-standard technique to assess liver histology, including steatosis, mixed inflammatory-cell infiltration, hepatocyte ballooning and necrosis, glycogen nuclei, Mallory's hyaline and fibrosis.

1.1.2 Overview of the Role of the Liver: Carbohydrate and Lipid Metabolism

The liver plays a prominent role in the regulation and metabolism of carbohydrates, lipids and proteins. The metabolism of these products are required for numerous biochemical functions and whole-body homeostasis, and allow the liver to produce substrates, hormones and nutrients.[13]

The liver also plays an important role in the storage of glucose following eating and the breakdown of stored glycogen released as glucose into the blood stream during fasting. Upon ingestion, glucose is transported to the liver via the hepatic portal vein and hepatocyte membrane through glucose transporters (specifically glucose transporter 2). Glucose is taken up and phosphorylated in the hepatocyte by the enzyme glucokinase to form glucose-6-phosphate (G6P). One pathway for the synthesised G6P is that of glycogen synthesis, activated in the liver following secretion of insulin by the pancreas and intestinal glucose supplied by the hepatic portal vein.[14] Glycogen synthesis is stimulated by insulin and glucose when they activate main regulatory enzyme, glycogen synthase and block the enzyme glycogen phosphorylase in order to supress glycogen breakdown. These mechanisms will result in glycogen storage in the liver. Under fasting conditions, glycogen stored in the liver will be broken down and released into the bloodstream as glucose in response to a low blood glucose concentration. Whereas in diabetes, fasting hyperglycaemia occurs via endogenous glucose production due to insulin resistance (IR) and postprandial hyperglycaemia resulting from the inability to store glucose as glycogen after a meal.[15] It is estimated that approximately 80% of individuals with diabetes have excess accumulation of glycogen in the liver, and 40-70% of these people also have hepatic fat accumulation.[16]

When excess amounts of fat are transported to the liver, elevated hepatic synthesis, reduced oxidation, and limited excretion of fat results in the accumulation and storage of hepatic triglyceride.[17] Metabolic dyslipidaemia is defined as circulating triglycerides being elevated above 1.7 mmol/L and low high-density lipoprotein (HDL) cholesterol (below 1.03 mmol/L in men and below 1.29 mmol/L in women).[18] Metabolic dyslipidaemia often occurs alongside hepatic steatosis and increased hepatic lipogenesis triggers the aforementioned outcomes. In obese and insulin resistant mice, it has been shown that insulin continues to stimulate lipogenesis while failing to suppress gluconeogenesis.[19] The mechanism by which the pathogenesis of fatty liver and hypertriglyceridemia occurs in these mice is suggested to be a selective post receptor defect in hepatic insulin action.[19] In humans, IR coincides with impaired muscle glycogen synthesis resulting in larger proportions of ingested energy being diverted to hepatic de novo lipogenesis (DNL) and increases in plasma triglyceride concentrations. This is accompanied by increases in triglyceride synthesis, decreased levels of HDL cholesterol and increased production of very low density lipoprotein (vLDL) cholesterol.[20] For individuals with insulin resistance it is these predisposing factors that contribute to the development of NAFLD and risk of CVD.

Under normal conditions, ingestion of dietary glucose stimulates insulin secretion from the pancreas (**Figure 1.1A**). The insulin travels to the liver via the portal vein where it will produce two key actions at the gene transcription level. In the first process, insulin stimulates phosphorylation of transcription factor fork head box protein O1 (Fox01), which activates gluconeogenesis. Phosphorylation of Fox01 stimulated by insulin presents the transcription factor from entering the nucleus, thus downregulating genes which are required for gluconeogenesis, particularly phosphoenolpyruvate carboxykinase and G6P.[21] The outcome of this pathway is reduced hepatic glucose output, which assists the maintenance of low blood glucose concentrations. In the second process, the sterol regulatory element-binding transcription factor 1 (SREBP-1c) is activated by insulin which increases transcription of genes including acetylcoenzyme A carboxylase and fatty acid synthase. These two genes are required for fatty acid and triglyceride biosynthesis. Triglycerides produced are secreted in vLDL which delivers the triglyceride to adipose tissue to be stored and skeletal muscle for combustion. In adipose tissue, insulin enables the uptake of vLDL-derived fatty acids and improves the quantity of lipoprotein lipase on the surface of endothelial cells.[21] These typical conditions are compromised in individuals who are obese, insulin resistant or have type 2 diabetes mellitus (T2DM). In individuals with NASH, Fox01 determines the gluconeogenic capacity of the liver, in that increases in expression and transcription may emphasise inflammation and disrupt lipid metabolism, dysregulating hepatic gluconeogenesis and contributing to long-term effects observed in pathological states.[22]

In individuals with compromised insulin signalling, such as those with T2DM, IR is induced via the Fox01 pathway in the liver and despite the very high insulin levels, mRNAs for phosphoenolpyruvate carboxykinase and G6P remain high and gluconeogenesis continues.[22] The SREBP-1c pathway maintains insulin sensitivity and elevated levels of nuclear SREBP-1c therefore enhance fatty acid synthesis causing excess triglycerides to accumulate in the liver. Excess triglycerides are secreted in vLDL, raising plasma triglyceride concentrations and increasing fatty acid deposits in the liver, muscle and adipose tissue.[19] These processes are shown in **Figure 1.1B**. This cascade of effects will worsen an insulin-resistant state and the total result is the classic T2DM triad, also the key components of the Metabolic Syndrome (MetS); hyperglycaemia, hyperinsulinemia and hypertriglyceridemia.

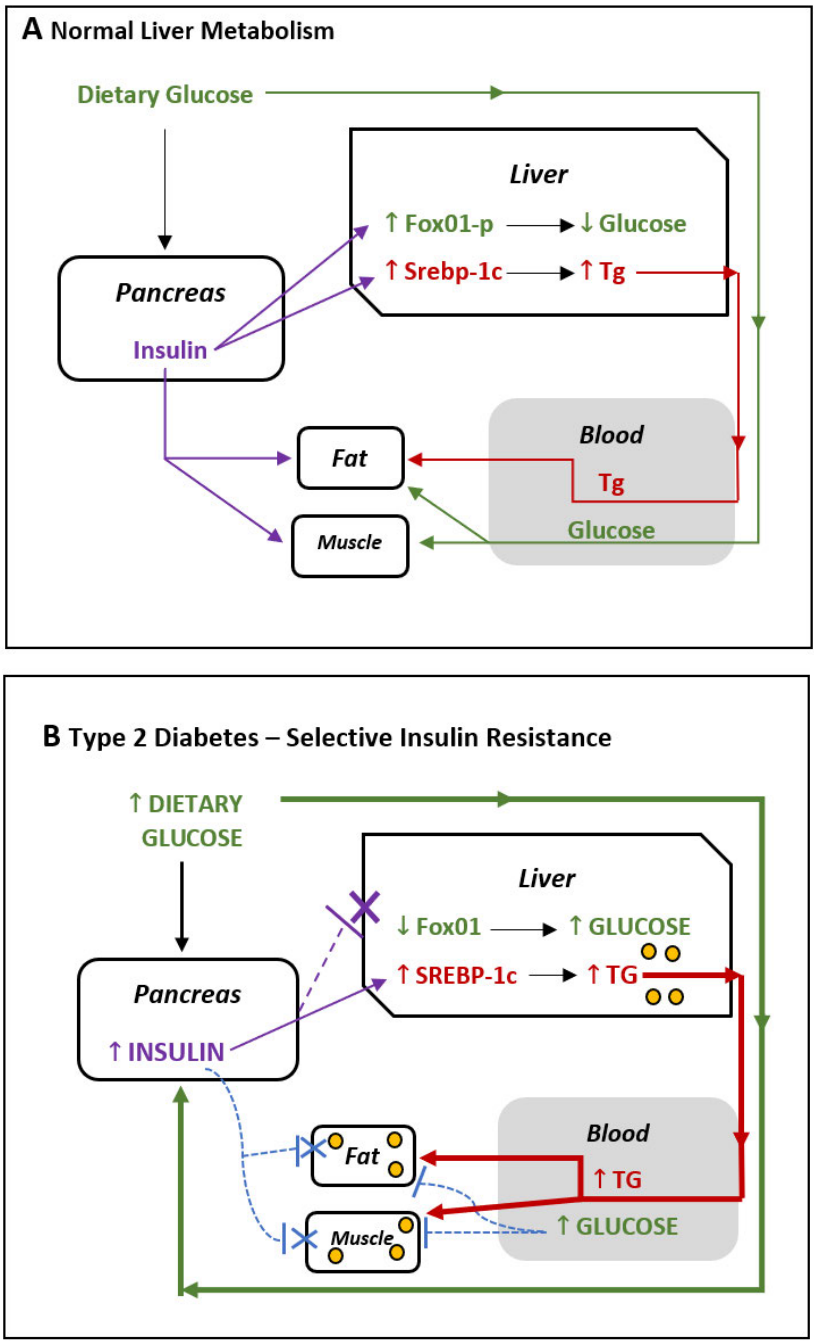


Figure 1.1. Model illustrating (A) normal response of the liver to a glucose load and (B) selective insulin resistance in liver with type 2 diabetes mellitus. *Adapted from Brown and Goldstein (2008)*[21]

1.1.3 Epidemiology

1.1.3.1 Prevalence of NAFLD

NAFLD is now recognised as the leading cause of chronic end-stage liver disease in the Western world, with prevalence rates quickly rising in developing countries.[23] Currently, the global prevalence estimate of NAFLD in adults is 25% (95% CI, 22.10-28.65) and its subtype, NASH, affects up to 5% (95% CI, 1.5-6.45) of the population.[24, 25] Global epidemiology reports show that rates of NAFLD are most prevalent in the Middle East (32%), South America (31%), Australia

(30%), Asia (27%), the United States (US)(24%) and Europe (23%).[25] Whereas, NAFLD is less common in Africa (14%) [26] or rural India (9%).[27] Consdiering the asymptomatic nature of NAFLD and diagnostic challenges, these rates are likely to be an underestimate. Researchers have estimated that more than 80 million indiviuals in the US and 52 million indiviuals in European countries are affected by NAFLD.[24, 28] In 2013, an executive and economic summary reported that NAFLD was the most prevalent liver disease in Australia affecting up to 5.5 million Australians, including 40% of the adult population over 50 years old.[29] This report stated that NAFLD was the second leading cause of mortality by liver disease in Australia, an estimated 2,264 deaths in total.[29] In more recent report published in 2020, the burden of NAFLD in Australia was projected to increase by 25% (5.5 million cases to 7.0 million cases) by 2030. Incidentally, NAFLD-related liver deaths were estimated to increase by 85% (1,900 deaths to 3,500 deaths) by 2030.[30]

1.1.3.2 Prevalence of Non-Obese and Lean NAFLD

Since the identification of the "metabolically unhealthy lean" phenotype, increasing recognition of "lean" or "non-obese" NAFLD has developed in individuals whose BMI, by classification of the World Health Organisation criteria, is non-obese.[31] Lean NAFLD, first recognised in Asian populations, encompasses hepatic steatosis and a metabolically unhealthy state with visceral obesity, in the absence of systemic obesity. In 2020, a systematic review and meta-analysis of 33 observational studies from 14 countries with a total of 205,307 individuals found that the global prevalence estimate of lean NAFLD in the overall population was 4.1% (95% CI, 3.4-4.8%), while the global prevalence of NAFLD in the lean population was 9.7% (95% CI, 7.7-11.8%).[32] Asia had the highest prevalance of lean NAFLD (4.8%, 95% CI, 4.0-5.6%), followed by North America (3.1%, 95% CI, 2.3–3.8%), Oceania (3.5%, 95% CI, 3.1–3.8%) and Europe (2.2%, 95% CI, 0.2-4.2%). Moreover, the global prevalence of metabolic comorbidities in lean NAFLD was 0.6% (95% CI, 0.4–0.9%) for T2DM, 1.4% (95% CI, 1.0–1.9%) for the metabolic syndrome, 2.8% (95% CI, 1.9–3.7%) for dyslipidemia and 2% (95% CI, 1.6–2.4%) for central obesity.[32] These results indicate an increase in the prevalence of lean NAFLD, particularly in Asian and Western countries,[33] higher in lean individuals with coextisting metablic risk factors and/or complications. For this patient group, lifestyle modifications such as diet which don't rely on weight loss to elicit metabolic or other health benefits are especially important.

1.1.3.3 Incidence and trends of NAFLD

The incidence of NAFLD in the general population has not been investigated often or thoroughly, largely due to the lack of sophisticated non-invasive methods of NAFLD diagnosis. The few studies which have reported incidence of NAFLD use ultrasonography, sometimes coupled with raised liver enzymes, which is considered a crude, imprecise and highly insensitive means of quantification.[34] The incidence of NAFLD varies world-wide, though altogether has been increasing dramatically over time. In Israel, incidence estimates range from 28 per 1000 persons/year (95%

CI, 19.34–40.57), in England estimated incidence rates were 29 per 100,000 persons/year and in Asia incidence estimates were approximately 52 per 1000 persons/year (95% CI, 28.31–96.77).[35-37] A population-based study from Minnesota (US), indicate a 5-fold increase in NAFLD incidence since 1997. Incident fatty liver has been strongly associated with anthropometry and biochemical factors of the MetS including weight gain or obesity, insulin resistance, hypertension and unfavourable lipid profile.[34] Poor dietary intake and nutritional status was also closely connected to incidence of NAFLD.[34, 38]

1.1.3.4 Health burden of NAFLD

Approximately 30-40% of those with NAFLD develop non-alcoholic steatohepatitis (NASH) and 40-50% develop hepatic fibrosis,[39] increasing the potential for progression to cirrhosis, liver failure and HCC.[40] In fact, the burden of NAFLD-related cirrhosis is currently twice as high as cirrhosis caused by chronic hepatitis C (CHC) in the USA.[41] Previously CHC-related cirrhosis was the leading cause of liver transplantation, though in recent years it's incidence has decreased and cirrhosis as a result of NAFLD has surpassed CHC as the leading indication for liver transplantation.[41] Prediction models have shown that even if the prevalence of obesity stabilises in Australia, NAFLD-related morbidity and mortality will increase due to the ageing populaion.[30] Between 2019 and 2030, more than 1.4 million persons ≥65 years will be at greater risk for advanced liver disease.[30] Cases of incident primary liver cancer are predicted to rise by 75%, from 420 (280-660) cases in 2019 to 730 (480-110) cases in 2030; modeled data was compared with reported estimates for 2005–2015, which was the most reliable data available.[30] Epidemiological studies have shown that NAFLD is present in up to 80-90% of obese individuals and 75% of individuals with type 2 diabetes mellitus (T2DM).[26] With approximately 1.2 million individuals dignosed with T2DM in Australia in 2015 and an approximate 20% of cases undiagnosed, by 2030 there will be an additional 2.2-3.0 million cases of diabetes.[30] Physicians are urged to consider individuals with T2DM a high-risk group. NAFLD is also typically associated with the male sex, increasing age, increased liver enzymes, and cardiometabolic alterations consistent with the metabolic syndrome (MetS).[42] Clinically, NAFLD is considered the hepatic manifestation of MetS and the leading predictor of cardiovascular disease (CVD). Indeed, cardiovascular complications are the leading cause of mortality among the NAFLD population.[43]

1.1.3.5 Economic Burden of NAFLD

The increasing health burden of a disease is ensued by the consequential economic burden of the disease. It has been predicted that the annual financial burden of NAFLD associated with the 64 million individuals affected by NAFLD in the US and the 52 million individuals affected by NAFLD in European countries was US$103 billion ($1,613 per patient) and €35 billion (from €354 to €1,163 per patient), respectively.[28] The total health burden of liver disease in Australia was estimated to be AUD$432 million, however the health cost of NAFLD itself was unidentified.[29]

Direct and indirect costs related to NAFLD are anticipated to rise in parallel with increasing prevalence of obesity and the development of new cases of NAFLD.[44] While the financial burden of NAFLD has not been forecasted in Australia, the USA and Europe have estimated an expected 10-year burden of $1.005 trillion and €334 billion, respectively.[28]

The main question that arises from forecasting the future health burden of NAFLD and NAFLD-related comorbidities is whether the significant cost of screening and management of early stages can be justified. The cost-utility analysis of NAFLD screening is hindered by the lack of high-quality evidence for effective and sustainable management strategies during the early stages of disease and the variability of inexpensive, non-invasive markers for diagnosis of NAFLD. The UK National Institute for Health and Care Excellence (NICE) NAFLD Guideline Committee do not recommend steatosis testing due to the ambiguity in cost effective and accurate tests and lack of strong clinical evidence.[45] Whereas the EASL–EASO–EASD 2016 guidelines and the UK NAFLD Guideline Committee recommend screening of patients with NAFLD for advanced fibrosis and cirrhosis using biomarkers, NAFLD fibrosis score and transient elastography or acoustic radiation force impulse imaging.[45, 46] Effective strategies for early detection and management of NAFLD and investment of resources at the subclinical level of disease should be main factors in analysing cost-benefit analysis, as they pose added risk of adverse health outcomes of NASH.[47] Moreover, a more established evidence-base regarding the management of early stage disease may help to justify screening. Currently, there are no high quality therapeutic management strategies in place, particularly in non-Mediterranean countries, which may be able to justify the cost of screening and in turn effective management of patients.

1.1.4 Risk Factors and Clinical Features of NAFLD

1.1.4.1 Age

The burden of chronic disease is known to increase with ageing, and age is indeed considered an independent, non-modifiable risk factor for NAFLD.[48] The structural integrity of the liver changes substantially over time, reducing metabolic function and detoxification properties.[49] The prevalence of NAFLD has been shown to increase in aging populations and age has been associated with more progressed liver damage and increased mortality.[50-52] A study conducted by Frith et al. (2009) divided a group of biopsy-proven NAFLD patients into older (≥60y), middle-aged (≥50y to <60y) and younger (<50y) groups, finding a positive correlation between age and the prevalence of NAFLD and fibrosis.[53] In the middle-aged and older groups of patients with NAFLD, there was a higher prevalence of risk factors such as hypertension, hyperlipidaemia and diabetes.[53] Another study in a geriatric rehabilitation hospital reported a prevalence rate of 46% NAFLD in the population, which is higher than the general population.[29, 54] This study also found that patients in the geriatric hospital were free of risk factors or comorbidities including CVD, the MetS or cirrhosis, suggesting that NAFLD pathogenesis may be altered in geriatric populations.[54] Many

studies have reported the association between older age and the increased risk of developing progressed liver problems such as NAFLD/NASH-fibrosis, HCC and T2DM.[55-57] In fact, a finding of the study by Frith and colleagues mentioned above, stated that younger age was associated with higher level of ALT and hepatic steatosis, whereas older age was associated with significantly higher grades of hepatic fibrosis and cirrhosis.[53] Overall, the age-specific pattern of NAFLD has not been fully defined. While there are pathophysiological changes occurring in the liver with aging that can ultimately affect hepatic blood flow and lipid metabolism,[49] it is unknown whether higher prevalence of NAFLD in older people is due to age itself or the duration of disease.

1.1.4.2 Sex

Sex differences have been reported in the prevalence of NAFLD. Initially it was estimated that NAFLD was more prevalent amongst females than males, however this finding lacked empirical evidence. In more recent data from larger scale, population-based studies, the majority of individuals with NAFLD tended to be males rather than females. A study into the medical health check-ups of 26,527 subjects in Asia showed that NAFLD was prevalent in 31% of men and 16% of women.[58] Another study of clinicopathological profiles of Indian medical patients reported that the majority of NAFLD cases were men.[59] Further to this, analysis from The Third National Health and Nutrition Examination Survey (NHANES III) showed that NAFLD was more prevalent in men than women at all ages, except for <30 years for which rates were similar.[60] Male sex has been associated with higher ALT and histological NASH, as well as hepatic fibrosis and all-cause mortality in patients diagnosed with NAFLD.[51, 61, 62] An interesting finding of sex-specificity is that females tend to be more likely to develop NAFLD as they age, likely due to sex hormone changes following menopause. The role of oestrogen as a protective factor in young women, is reduced following menopause, along with changes in IR which can lead to susceptibility to NAFLD.[63] Considering the multi-factorial interaction between age, sex and NAFLD, additional large, population-based cohort studies are required to gain additional understanding of this topic.

1.1.4.3 Ethnicity

Ethnic/racial disparities have been widely reported in the prevalence of NAFLD. Typically, studies in the United States show that Hispanics had the highest prevalence of NAFLD, followed by non-Hispanic Whites and the lowest rates were reported in African Americans.[61, 64, 65] Confirming previous findings, a recent systematic review of population-based cohorts and smaller high-risk groups investigated differences in prevalence, severity and outcomes based on ethnicity. Authors found the same trends in prevalence between Hispanics, non-Hispanic Whites and African Americans, although differences between groups were smaller high-risk cohorts than population-based cohorts.[66] They also found that while prevalence of NAFLD and risk of NASH was highest in Hispanics and lowest in African Americans, there was no significant difference between the ethnic groups in relation to the proportion of individuals with advanced fibrosis.[66] NAFLD is

becoming more prevalent in Asian populations, even in lean patients with "normal" BMI.[67] Current literature does not characterise the severity and prognosis related to NAFLD between ethnic/racial groups, however studies are needed to define why disparities exist and how it affects the progression of NAFLD. Cultural, environmental and socioeconomic factors are likely contributors to the effect of ethnicity on dietary and lifestyle habits, beliefs and accessibility to health care. The risk of NAFLD extends through these areas.

1.1.4.4 Genetics

The notion that there is an underlying genetic susceptibility to NAFLD has been proposed and increasingly accepted with additional studies emerging. Genomic research connects genotypes with epidemiology of disease providing important evidence for disease origin, characteristics and predictors of disease. Familial clustering of NAFLD was first observed in twin and other hereditary studies, and population-based studies helped to identify differences in susceptibility, progression and severity of NAFLD.[68-70] Familial studies show that heritability of NAFLD occurs in approximately 27% of cases.[71] Single nucleotide polymorphism (SNP) variants are used as potential genetic markers for many diseases. In NAFLD, SNPs of candidate genes have been identified from larger genome-wide association studies (GWAS) and associated with insulin resistance, lipid metabolism, inflammation, oxidative stress and liver histology. One of the most commonly studied gene variants in NAFLD populations is the I148M allele of patatin-like phospholipase domain containing protein 3 (PNPLA3) gene, which encodes adiponutrin and is strongly associated with accumulation of fat in the hepatocyte.[72] Evidence indicates that GG homozygote carriers have a 73% higher hepatic fat content when compared with CC carriers, as well as a 3.2-fold higher risk of developing liver fibrosis.[73] The PNPLA3 I148M variant is more prevalent in Hispanics (49%) compared with European Americans (23%) and African Americans (17%). Importantly, the association between the PNPLA3 variant and hepatic fat appears to be independent of insulin resistance and serum lipids, and may modify response to diet and lifestyle factors such as obesity.[73] A joint effect of PNPLA3 and mutations in the glucokinase regulator (GCKR) gene have been reported, and GCKR is also associated with significant liver fibrosis and elevated serum lipid concentration.[74] A meta-analysis of GWA studies identified PNPLA3 and GCKR, as well as gene variants Neurocan and lysophospholipase-like 1, as significantly associated with increasing hepatic steatosis and histologic NAFLD, as determined by computed tomography (CT).[75] A number of genetic variants have been identified which may influence NAFLD susceptibility and progression including peroxisome proliferator-activated receptor-alpha (PPARα), peroxisome proliferator-activated receptor-gamma (PPARγ), apolipoprotein C3 (APOC3) and apolipoprotein E (APOE), however only a few of them have been validated and confirmed in several independent populations.[76]

According to current literature, there are multiple hypotheses about the mechanism of gene variants in NAFLD: (i) genes influencing hepatic FFA and TG; (ii) genes affecting insulin resistance; (iii)

genes influencing oxidative stress; (iv) genes influencing response to endotoxin; (v) genes influencing the release or effect of cytokines/adipokines; (vi) genes affecting severity of fibrosis; and (vii) genes which predispose to HCC.[77, 78] Gene variants influencing inflammation in NAFLD will be the primary focus of Chapter 6 of this doctoral thesis. This topic will be covered in detail in a latter section of this literature review (**Section 1.1.5**).

1.1.5 Common Metabolic Disorders related to NAFLD

IR is present in most individuals with NAFLD, clinically defined as the critical link between metabolic stress, visceral adiposity, and decreased cardiorespiratory fitness.[79] It is well-known that NAFLD has a strong association with IR not only in the liver but also in muscle and adipose tissue. Biologically, hepatic IR will result in elevated fasting blood glucose levels, while peripheral (muscle) IR will result in an increase in concentration of circulating FFAs worsened by adipose tissue IR inducing lipotoxicity by increasing the flux of FFA to the liver and other target tissues.[79, 80] IR is strongly associated with the development and progression of other metabolic abnormalities including pre-diabetes, T2DM and the MetS, all of which increase the likelihood of cardiovascular complications and/or cardiovascular disease (CVD)-related mortality. The notion that NAFLD may be an early mediator of atherosclerosis and could predict future CVD events is widely recognised and the importance of understanding links between diseases continues to be investigated.[81]

1.1.5.1 Obesity

Obesity is a key risk factor and characteristic of NAFLD and other chronic metabolic disorders. Inflammation emerges in the presence of obesity, primarily through inflammatory processes occurring in visceral adipose tissue and other metabolically active sites including the liver. Obesity is strongly associated with conditions such as T2DM, CVDs, hypertension and stroke, osteoarthritis, some types of cancer and overall mortality.[82] Obesity is reported to reduce life expectancy by up to 20 years.[83] The prevalence of NAFLD and severity of steatohepatitis increases with increasing BMI.[84] Studies have shown that individuals at "low-risk" of NAFLD were typically classified as "healthy" weight (BMI $\leq$25 kg/m^2), free of diabetes and had normal serum fasting glucose and ALT concentrations.[85] Data from multiple sources of liver histology analyses indicate that the prevalence rates of NAFLD are approximately 15% in non-obese individuals, 65% in those classified as class I and II obesity (BMI 30.0-39.9 kg/m^2) and 85% in morbidly obese individuals (BMI $\geq$40.0 kg/m^2).[86-89] From the same sources, the prevalence of NASH was approximately 3% in non-obese persons, 20% in the class I and II obese and 40% morbidly obese individuals.[86-89] A strong correlation exists between visceral adipose tissue – a measure of central adiposity – and NAFLD.[90] Visceral fat has a greater lipolytic potential than subcutaneous adipose tissue and is known to increase FFA flux, in turn elevating circulating triglycerides and potentially mediating IR.[84] In patients with NAFLD, those who are centrally obese tend to be insulin resistant compared to those who do not have central-obesity (i.e., lower proportion of visceral fat).[84] Another functional

difference between visceral and subcutaneous fat stores is the release of proinflammatory cytokines from visceral adipose tissue as opposed to subcutaneous tissue.[91]

1.1.5.2 T2DM and CVD

NAFLD represents a significant burden of disease for patients with T2DM, not only in prevalence, but also in severity of disease. It has been estimated that 70-75% of patients with T2DM have some form of NAFLD,[92] and NAFLD may develop and progress in patients with T2DM independent of the diabetes progression itself.[93] Traditionally, these studies were based on abnormal liver function tests which are a poor proxy marker of NAFLD however this association has now been identified using ultrasonography to examine hepatic steatosis. Two cross-sectional studies reported that the unadjusted prevalence of ultrasonographic NAFLD among individuals with T2DM was 69%, with NAFLD the most common cause (81.5%) of hepatic steatosis on ultrasound examination.[92, 93] Abdominal obesity, hypertriglyceridemia and a high-normal ALT level were independently associated with an increased risk of having NAFLD.[93] Another study, which consisted of 204 patients with T2DM identified 127 of their patients had fatty infiltration based on ultrasound. Of these 127 patients, 90 patients consented for liver biopsy which found that 87% of patients had NAFLD on histology, 62.6% of patients had steatohepatitis and 37.3% had fibrosis.[94] Another study found that individuals with NAFLD were 1.6 times more likely to develop T2DM than NAFLD-free persons over a 3-year follow up, highlighting the importance of large epidemiological studies and the need to better understand underlying mechanisms leading to disease development and progression.[95]

The pathophysiological mechanisms underlying the high prevalence of NAFLD and its unfavourable outcomes in patients with T2DM remain under debate. Aside from IR, NAFLD and T2DM share key pathophysiological features including chronic low-grade inflammation, increased oxidative stress and upregulation of pro-inflammatory cytokines.[96, 97] These underlying features are also known CVD-risk factors. When compared, patients with NAFLD were found to have a higher prevalence of coronary heart disease (CHD)(16.8 vs. 9.1%) and cerebrovascular disease (9.6 vs. 5.5%), than those without NAFLD.[93] In a separate study, the most common cause of death in 49 NASH patients followed up over ~4 years was CVD.[88] Further, Matteoni et al. (1999) found that CVD was the second most common cause of death in 132 NAFLD patients who were followed up for ~18 years, and rates equalled those of liver-related deaths in their population.[98] Such results are important in the implication and clinical treatment of NAFLD, though it is not presently known whether improving NAFLD will ultimately prevent the development of CVD.[43] Current screening strategies and health guidelines for individuals with diabetes[99, 100] and CVD[101] do not include individuals with NAFLD.

1.1.5.3 The Metabolic Syndrome (MetS)

The MetS is characterised as a cluster of cardiometabolic disturbances including impaired glucose tolerance (prediabetes), hyperinsulinemia, dyslipidaemia (increased triglycerides and decreased high density lipoprotein (HDL)-cholesterol), hypertension and central obesity.[102] Though this condition has been identified for over 80 years, multiple definitions exist without one being used universally. For the purpose of consistency throughout this doctoral thesis, the MetS will be defined using the widely known and commonly used National Cholesterol Education Program Expert Panel (NCEP) Adult Treatment Panel III (ATP III) criteria.[103] This definition was proposed in 2001[104] and modified by the American Heart Association and National Heart, Lung and Blood Institute (AHA/NHLBI) in 2005.[103] As detailed in the updated ATP III report, **participants with ≥3 of the following criteria were defined as having the MetS:**

- Central obesity: waist circumference ≥102 cm in men and ≥88 cm in women;
- Hypertriglyceridemia: ≥1.69 mmol/L;
- Low high-density lipoprotein (HDL) cholesterol: <1.04 mmol/L in men and <1.29 mmol/L in women;
- High blood pressure: ≥130/85 mmHg;
- High fasting glucose: ≥6.1 mmol/L.

Since there is no universal definition of the MetS, published prevalence rates vary for different populations making it difficult to assess the health impact and economic burden world-wide.[102] Using the NCEP criteria definition, the prevalence of the MetS was approximately 24% of the general population in the United States.[105] Rapidly rising rates of obesity will continue to drive prevalence of the MetS, with age, physical inactivity, ethnicity and genetics identified as predisposing factors.

Considering that most of the cardiometabolic disturbances which make up the MetS result from systemic and hepatic IR, it is not surprising that NAFLD is considered the "hepatic manifestation" of the MetS. In fact, at least 80% of individuals with the clustering of disease comorbidities manifesting as MetS have NAFLD and of these, up to 25% were diagnosed with NASH based on liver biopsy.[106] In one study, 88% of NASH patients met the criteria for the MetS, compared with 53% of NAFLD patients.[106] In an 11 year population-based cohort study conducted by Adams et al. (2009), patients with NAFLD and elevated liver enzymes were three times more likely to develop diabetes and 50% more likely to develop the MetS compared with the general population.[107] Another study found that patients with NAFLD had increased incidence of the MetS and NAFLD was an independent risk factor for the MetS.[108] This study also confirmed that risk factors associated with the MetS included IR, T2DM and hypertension.[108] Similarly, data from the Non-alcoholic

Steatohepatitis Clinical Research Network showed that prevalence of the MetS increased the likelihood of developing histologically confirmed NASH by 40%,[109] and was independently associated with higher all-cause mortality among NAFLD patients in the National Health and Nutrition Examination Survey database.[110] It is evident that IR plays a central pathophysiological role in the occurrence of metabolic abnormalities and coupled with a prolonged proinflammatory state may promote the development of the MetS, T2DM and NAFLD.

1.1.1 Clinical Presentation

Non-alcoholic fatty liver disease is considered to be an asymptomatic condition. Some patients have reported non-specific fatigue or weakness, and some experience slight right-sided abdominal pain.[111]

Liver function tests that have been performed for other medical reasons – often as part of a general health check or blood. Over two-thirds of individuals who present with unexplained abnormal liver function tests will be diagnosed with NAFLD in subsequent follow up, however the degree to which liver enzymes are elevated may not be related to disease severity.[112] Risk factors that are typically observed in patients presenting with NAFLD include: increasing age, BMI and central obesity, family history, sedentary lifestyle, poor diet, diabetes or insulin resistance and hyperlipidaemia.[44] Patients who present with one or more of these risk factors should be identified and followed up by treating physicians who are well equipped in the treatment and diagnosis of NAFLD.

1.1.2 From NAFLD to MAFLD

The terms non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) were first coined in the early 1980s when clinicians and pathologists observed similar histopathological features of alcoholic liver disease (ALD) in a series of patients who denied misuse of alcohol.[113, 114] At the time, the common features documented between NAFLD and ALD were steatosis, steatohepatitis and fibrosis, and understanding of the complex pathogenesis of NAFLD was limited. Over time, landmark studies by Day and James[115] and Marchesini et al. (2001)[116] have proposed inflammation, oxidative stress and insulin resistance as key drivers of NAFLD pathogenesis and evolution to NASH and these theories have been widely accepted and supported by subsequently published literature. Over the past two decades, rising rates of NAFLD have paralleled prevalence rates of obesity and diabetes[44] and numerous studies have illustrated metabolic dysfunction as a key driving feature of NAFLD.[117] In fact, NAFLD has often been termed the hepatic manifestation of the metabolic syndrome.

Recently, two new position papers have highlighted the urgent need for revised nomenclature surrounding the disease.[118, 119] In these papers, a group of highly distinguished experts propose the name NAFLD be changed to metabolic associated fatty liver disease (MAFLD) based on the clear metabolic underpinnings of the disease. The proposed name change is accompanied by a new

criteria in which evidence of hepatic steatosis must be present along with one of three features: (i) overweight or obesity; (ii) T2DM; or (iii) lean or normal weight with evidence of metabolic dysregulation (**Figure 1.2**).[120]

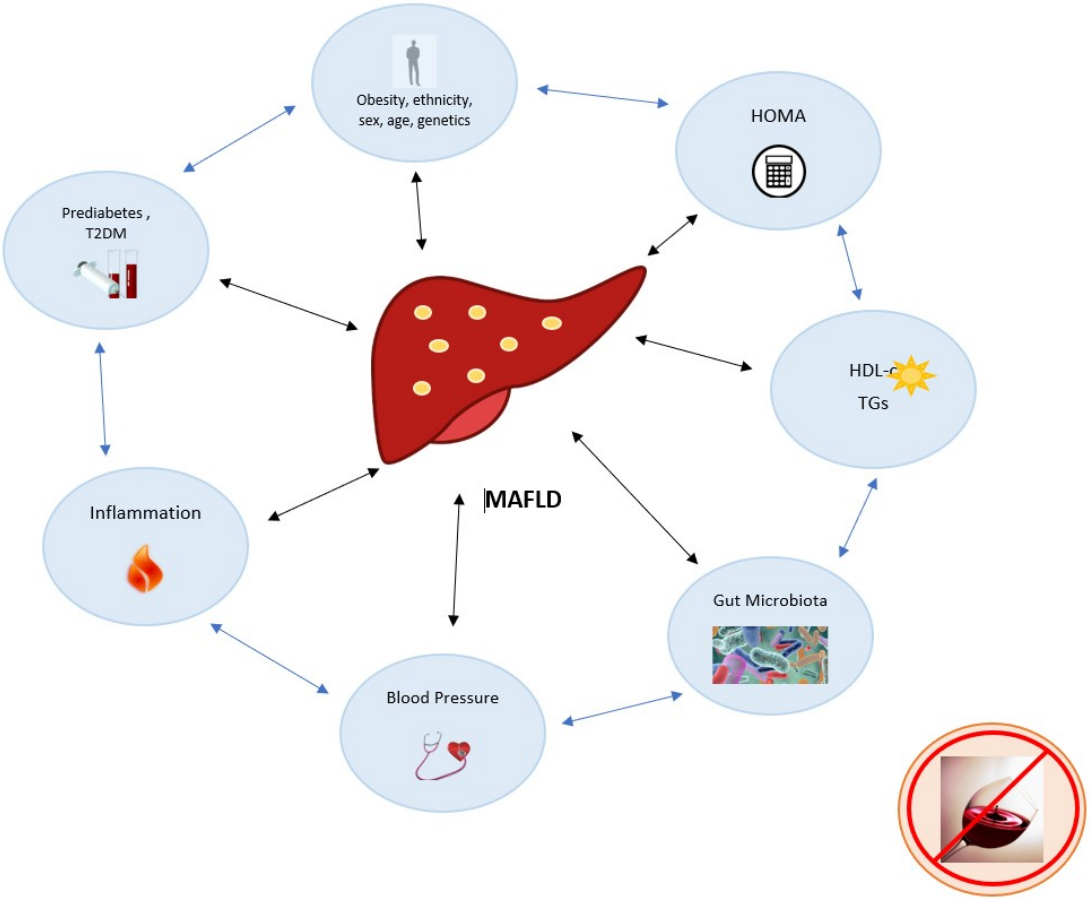


Figure 1.2. MAFLD: an 'old' new disease. The criteria for Metabolic Associated Fatty Liver Disease (MAFLD) include hepatic steatosis, accompanied by either: (i) obesity or overweight (based on BMI >25 kg/m^2 in Caucasian individuals and >23 kg/m^2 in Asian individuals), (ii) type 2 diabetes mellitus, or (iii) evidence of metabolic dysregulation. Of the following metabolic risk factors, at least two should be present: waist circumference ≥102/88 cm in white men and women or ≥90/80 cm in Asian men and women; inflammation with elevated hs-CRP; prediabetes; high blood pressure or specific pharmacological treatment; decreased levels of HDL-cholesterol; elevated levels of plasma triglycerides; and homeostasis model assessment (HOMA)-insulin resistance score ≥2.5. *Adapted from Tilg and Effenberger (2020)*[120]

This change is intended to challenge the current criteria which is based on the presence of >5% steatosis in the absence of significant or ongoing alcohol use and exclusion of other liver diseases. The new criteria propose the shift to a "positive" criteria set specifically for the diagnosis of MAFLD. Interestingly, the exclusion of other significant alcohol intake or other chronic liver disease is no longer a requirement for the diagnosis of MAFLD.[121] In addition to these main criteria, hepatologists will need to screen for metabolic risk factors, at least two of which must be required for diagnosis of metabolic dysregulation: waist circumference ≥102/88 cm in white men and women, respectively, or ≥90/80 cm in Asian men and women, respectively; prediabetes;

inflammation via elevated high-sensitive serum C-reactive protein level; elevated blood pressure or specific drug treatment; decreased HDL-cholesterol levels; increased plasma triglycerides levels; and HOMA-IR score ≥2.5.[118] Eslam et al. (2020) also suggest that the assessment and stratification of severity criteria for NAFLD be extended beyond the simple dichotomous classification of NASH versus non-NASH, as this may not capture the full spectrum of disease.[118] The disease process in MAFLD may be best described by the grade of activity and stage of fibrosis present which will improve case identification and changes in the underlying metabolic dysfunction or response to lifestyle or pharmacological interventions.[118]

In agreement with expert views put-forth in the position papers, Tilg and Effenberger (2020) suggest that an important clinical concept and "next step" in characterising metabolic disorders in NAFLD is the presence of low-grade inflammation.[120] The new criteria for NAFLD include documenting C-reactive protein levels, and whilst this marker is a well-established risk marker of cardiometabolic disorders in general, its specificity to the development and progression of NAFLD is largely unclear. Tilg and Effenberger (2020) highlight the lack of attention that inflammation has received in MAFLD even though it is a key underlying feature of the disease.[120] Patients who fluctuate between steatosis and steatohepatitis over relatively short time frames, and those with steatohepatitis that rapidly progresses to fibrosis which may also regress, suggests that inflammation could be chronic-relapsing or intermittent in MAFLD as it is in many other chronic inflammatory and liver diseases.[120] These changes might be overlooked in a liver biopsy. Understanding the association between inflammation and MAFLD is important to better understand the natural disease course and recognise that treatment strategies may need to focus on anti-inflammatory approaches.

There are still challenges to overcome in the renaming NAFLD as MAFLD, including defining 'metabolic health' in general and more specifically defining metabolic health classification of sub-groups (such as metabolically healthy obese) and sub phenotypes of NAFLD. Experts in the field recognise that additional initiatives are warranted to sub phenotype patients with MAFLD, which will assist the precision of patient management and enable effective pathways between primary care and outpatient liver clinics.[118] There is a need for diabetologists, hepatologists and dietitians to intensify their collaborative approach in treating patients presenting with fatty liver disease. Treatment options should be built on evidence-based guidelines and dietary recommendations, underpinned by the work of researchers and ideally derived from the results of high-quality clinical trials. Large epidemiological studies have furthered the understanding of the natural disease course, though the need for non-invasive, accurate screening and diagnostic tools remains.[120] The development and validation of techniques such as serum biomarkers of steatosis could replace costly imaging methods, and more importantly capture metabolically unhealthy lean patients who may otherwise not have been referred for further follow-up or ultrasonography.

The implications of this new nomenclature, disease definition and diagnostic criteria will impact patients and practitioners, influence health awareness and stigma, and affect funding and health policy.[121] There is growing evidence that health professionals may undertreat patients with NAFLD due to the inappropriate branding of the medical condition, limited acceptance of treatment options and suboptimal funding and resources,[121] and this undertreatment is evidenced in the growing number of high-risk individuals presenting to outpatient liver clinics.[47] The negative consequences of the misnamed disease are apparent; therefore, it is time to reframe the disease criteria, definition and name in order to develop more effective and durable treatments. The proposed nomenclature, disease definition and diagnostic criteria are novel and practical. Future research should aim to consistently and repetitively validate the criteria in a clinical setting to confirm the feasibility of its use in the recruitment of participants to clinical trials and most importantly, to utilise in routine clinical practice.[118]

The present doctoral research was conducted prior to proposal of the new nomenclature, disease definition and diagnostic criteria, and therefore this doctoral thesis will refer to the disease as NAFLD and utilise its classic nomenclature, disease definition and diagnostic criteria.

1.1.3 Pathogenesis of NAFLD

The development and progression of NAFLD is recognised as complex and multifactorial. Although its pathogenesis remains poorly understood well-known and accepted hypotheses have been identified. From a pathological point of view, the first step involves excessive lipid accumulation in the liver which is mainly a result of a high consumption of dietary fat and/or calories, increased visceral adipose tissue (VAT) lipolysis and increased hepatic de novo lipogenesis (DNL) activation.[6] In a small study in human participants which used isotope labelling to identify the representation of each of these mechanisms, 59% was attributed to excessive free fatty acid (FFA) flux from VAT to the liver, 26% through DNL and 14% due to dietary fat and/or calories consumed.[122] Along with the expansion of adipose tissue, low-grade inflammation and IR also increase the rate of lipolysis. IR tends to drive metabolic complications in patients with NAFLD and is the main risk factor associated with co-morbidities including the MetS and T2DM.[123, 124] The progression of NAFLD is inconsistent and varies between patients. In some patients, NAFLD will progress to NASH and/or advanced liver disease rapidly whereas in others it may never progress to NASH.[6] It is acknowledged by clinicians and researchers that hepatic steatosis should be targeted in the early onset stages so that disease progression and potential co-morbidities can be prevented.

Initially it was thought that the progression of NAFLD to NASH involved two-hits; the ‘first hit’ driven by IR inducing lipid accumulation in the hepatocytes and increasing the vulnerability of the liver to further insults, after which the “second hit” will promote hepatic injury in the form of inflammation and fibrosis.[115] In recent years, this theory is viewed as over-simplistic given the complicated nature of NAFLD, where multiple parallel factors have been identified to

synergistically implicate disease development and progression to likely genetically predisposed individuals.[17] The evolution of the "two-hit hypothesis" to a "multiple-hits" hypothesis was first proposed by Tilg & Moschen in 2010,[125] who implied that alongside metabolic insults, extrahepatic tissues, environmental and genetic factors play an important role promoting liver inflammation (**Figure 1.3**). Dietary and environmental factors are implicated in the development of NAFLD and NASH, as they increase serum fatty acids and cholesterol, adipocyte proliferation and promote IR and changes in the intestinal microbiome. IR also acts on the development of hepatic steatosis by increasing DNL and adipocyte lipolysis, impairing adipose tissue function and promoting abnormal release of inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[17] A consequent influx of fatty acids to the liver results in hepatic fat accumulation in the form of triglycerides, while elevated levels of FFA, free cholesterol and other lipid metabolites induce mitochondrial dysfunction with oxidative stress.[125] The production of reactive oxygen species activates endoplasmic reticulum stress mechanisms which result in inflammation of the liver. An altered gut microbiota produces additional FFAs in the bowel, promoting intestinal permeability and fatty acid absorption and releasing inflammatory cytokines TNF-α and IL-6 via the activation of inflammatory pathways.[126] In addition, an underlying genetic predisposition to disease or epigenetic modifications is thought to affect the degree of hepatocyte steatosis and liver inflammatory environment in NAFLD and/or NASH patients. Thus, it is now recognised that a combination of these genetic, environmental, external and intracellular events, as well as inflammation preceding or in conjunction with steatosis, lead to the development of NAFLD and progression of NASH respectively.[17]

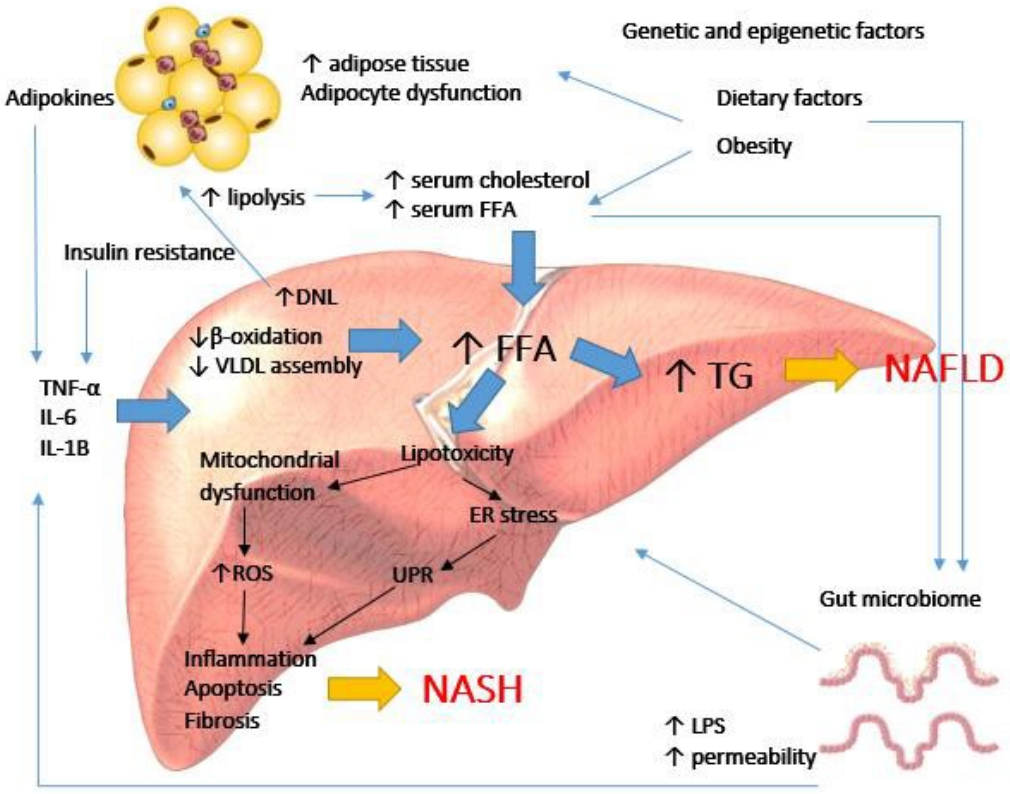


Figure 1.3. The Multiple Hit Hypothesis for the development of NAFLD. *Adapted from Buzzetti et al. 2016.*[17]

Reprinted with permission from George, E. S. (2017). *A Mediterranean diet for the management of non-alcoholic fatty liver disease* (Doctoral thesis, La Trobe University, Melbourne, Australia). Retrieved from http://hdl.handle net/1959.9/564467

1.1.4 Inflammation and NAFLD

Chronic low-grade inflammation is a common, yet not fully understood feature of NAFLD, which alongside other factors may further drive the disease along the spectrum of NASH fibrosis and cirrhosis. A mutual positive feedback process exists between IR and inflammation in the presence of lipotoxicity, termed the "vicious circle".[127] The uptake of FFAs from visceral adipose tissue and/or excess dietary intake accumulate in the liver and promote inflammation through the hepatic activities of nuclear factor-kappa B (NF-κB), and its upstream activator kinase-β (IKK-β). NF-κB is a sequence-specific transcription factor important for regulating the transcription of a wide range of inflammatory responses and IKK-β is responsible for its activation during acute inflammation.[128] Both NF-κB and IKK-β are involved in the aetiology of IR, though the cell types and mechanisms through which they are modulated are unknown.[129] Two studies in rodent models have shown that a high-fat diet was associated with increased NF-κB activity in the liver and increased hepatic expression of proinflammatory cytokines including TNF-α, IL-6, and IL-1β, and activation of Kupffer cells.[130, 131] Pro-inflammatory gene expression, Kupffer cell activation and hepatic and systemic IR responses to the high-fat diet were all able to be attenuated by hepatocyte-specific NF-κB inhibition, consistent with known inhibitory effects of TNF-α, IL-6, and IL-1β on insulin

signalling. The role of c-Jun N-terminal protein kinase (JNK/JNK1), Protein kinase C (PKC) and suppressors of cytokine signalling (SOCS) pathways are involved in regulating IR and models have shown JNK activity to be increased in the liver, muscle and adipose tissue probably in response to increased FFA and TNF-α.[132, 133] In an animal model, loss of JNK1 prevents IR in both genetic and dietary pathways of obesity and a knockdown of liver-specific JNK1 has been shown to decrease circulating levels of glucose and insulin.[134] Thus, the role of JNK1 in the development of IR is recognised though human models of JNK signalling have not yet been widely studied.

Obesity promotes a chronic inflammatory response in NAFLD via multiple mechanisms, including abnormal cytokine production, increased synthesis of acute-phase reactants and activation of inflammatory signalling pathways.[135, 136] Adipose tissue acts as an endocrine organ to produce circulating and systemically active cytokines, and adipocytes also containing a high amount of macrophages which comprise an additional source of soluble mediators (**Figure 1.4**). Macrophages can be characterised by their phenotype, M1 and M2. M1 macrophages produce pro-inflammatory cytokines such as TNF-α and IL-6, in contrast M2 macrophages produce anti-inflammatory cytokines such as IL-10 and adiponectin.[137] Obesity results in the accumulation of liver fat (hepatic steatosis), and the liver also begins to produce various inflammatory markers.[136] Obesity related-IR and chronic inflammation are key pathogenic factors to NAFLD/NASH, though it remains unclear which develops first.

1.1.4.1 Cytokines in NAFLD

TNF-α

The first study to find an association between obesity, expression of pro-inflammatory cytokine TNF-α and insulin action was published in 1992 by Hotamisligil and colleagues.[138] They found that adipocytes directly expressed TNF-α in rodents, which led to the concept that obesity had a role in inflammation.[138] Supporting evidence emerged for the role of TNF-α in IR, a study of mice lacking TNF-α or TNF receptors resulted in improved insulin sensitivity in dietary and genetic models of obesity.[139] Human studies paralleled the findings of animal models, multiple studies finding that increased expression of TNF-α was present in adipose tissue of obese individuals which subsequently decreased with weight loss.[140, 141] Elevated liver expression of TNF-α has been reported in individuals with NAFLD, and increased expression of TNF-α via TNF type 1 receptor have been significantly correlated with severity of NAFLD and the degree of fibrosis.[142] Similar studies have reported a positive correlation between the degree of liver fibrosis and TNF-α expression in NAFLD,[143] as well as increased TNF-α expression, liver and adipose tissue in patients with more severe NASH fibrosis, compared to those with less or non-existent fibrosis.[142]

Animal studies have administered anti-TNFα antibodies to rats with high-fat-diet-induced IR, finding that antibodies improved insulin signalling and reversed hepatic steatosis.[144] This finding indicated that neutralisation of pro-inflammatory cytokine TNF-α reduces inflammation in the liver,

steatosis and/or fibrosis, and mediates insulin. In humans, two clinical studies using an antagonist and an anti-TNF-α antibody did not find an improvement in insulin sensitivity.[145, 146] Although animal models have provided the potential therapeutic target of TNF-α inhibition, in humans, the role of TNF-α in IR and NAFLD requires further investigation.

Interleukin-6 (IL-6)

Interleukin-6 is a pleiotropic cytokine with a complex role in the pathogenesis of NAFLD, with many functions remaining unclear. The functional role of IL-6 is to activate cells, including immune cells, hepatocytes, stem cells, and more, to serve biological functions that will induce inflammation, modulate immune response or support oncogenesis or haematopoiesis.[147] Initially, IL-6 was thought to be hepatoprotective; capable of lowering hepatic steatosis, oxidative stress and preventing mitochondrial dysfunction.[148] However, IL-6 also has a key role in the acute phase response which facilitates the synthesis and release of several acute phase proteins, including C-reactive protein (CRP); an established marker of inflammation.[147] IL-6 is upregulated by TNF-α and has been positively correlated with systemic levels of CRP in morbidly obese patients, and is therefore suggested to play an indirect deleterious role in the pathogenesis of NAFLD.[147] Serum IL-6 has been found to be higher in patients with NAFLD[149] and increasing levels were observed alongside increases in NAFLD severity and fibrosis.[150, 151] Moreover, in patients with NASH, liver IL-6 expression was found to correlate with degree of inflammation and hepatic IR.[149] It should be noted, however, that the role of IL-6 in IR remains unclear.

Evidence for the role of IL-6 in IR was initially observed in obese mice that were treated with anti-IL-6 antibodies, resulting in improved insulin sensitivity.[152] One study of patients who underwent bariatric surgery to induce weight loss had significantly decreased IL-6 and improvements in IR after surgery.[153] While IL-6 has been considered a predictor for NAFLD and CVD, results of clinical studies investigating serum IL-6 concentrations are inconsistent.[136, 150, 154] Interpretation of experimental results is further complicated as IL-6 activity is influenced by other signalling pathways, cytokines and hormones.[150] It also remains indefinite as to whether IL-6 is primarily synthesised by the liver or produced by adipose tissue in NAFLD, which limits understanding of the physiological function of the cytokine.[136, 150]

CRP

C-reactive protein is a well-known acute phase reactant and marker of inflammation in chronic disease and infection, predominantly produced by the liver, but may also be released from adipose tissue.[155] The role of CRP in the development of atherosclerosis and CVD is well-established,[156, 157] though its role in the development and progression of NAFLD is less clear. Some studies have reported high CRP levels in patients with NAFLD compared to healthy controls,[158, 159] while others have found no difference between CRP in obese individuals with and without NAFLD.[160] In middle-aged participants with high concentrations of CRP, severity of NAFLD was an independent risk

factor for the development of CVD.[161] Serum CRP is significantly correlated with liver steatosis and severity of NAFLD, independent of body mass index (BMI), and therefore has been proposed as an obesity-independent marker of NAFLD.[155, 162] Whilst CRP may be representative of low-grade inflammation in the liver,[155] it is not an established marker of steatosis or severity of NAFLD.[155]

1.1.4.2 Adipokines

Adiponectin

Adiponectin is an important adipokine, most abundantly produced by adipocytes, though also expressed by skeletal muscle cells, cardiac myocytes and endothelial cells.[163] Adiponectin levels are reduced in obese individuals and in patients with T2DM, or IR states.[127, 136] Adiponectin contains insulin sensitising properties, via activation of AMP-activated protein kinase in the liver and muscle which leads to an increase in fatty acid oxidation, glucose uptake by muscle and suppression of gluconeogenesis in the liver.[164] Transcription of adiponectin in an adipocyte cell line is supressed by TNF-α, which provides explanation for lower concentrations of serum adiponectin observed in obese individuals.[165] Further, circulating pro-inflammatory mediators such as IL-6 regulate the expression of adiponectin by supressing transcription and translation in adipocytes.[166] Adiponectin synthesis is induced by weight loss and activation of peroxisome-proliferator-activated receptor-γ (PPARγ).[163]

Lower levels of serum adiponectin have been observed in patients with NAFLD and NASH, compared to healthy controls.[167] Hypoadiponectinemia has been identified as an independent risk factor for the development of NAFLD and liver dysfunction, suggesting that adiponectin deficiency is important in the accumulation of intrahepatic fat and not simply for development of IR.[168] Studies have reported that adiponectin expression is decreased by 20-40% when liver damage progresses from simple steatosis to NASH.[168] Adiponectin had an area under the curve of 0.765 and sensitivity and specificity of 68% and 79%, respectively, for identifying early-stage NASH with a cut-off value of ≤4.0 μg/mL.[169] Accumulating evidence shows that adiponectin can predict steatosis grade and severity of NAFLD, but not the severity of fibrosis.[10] Although adiponectin has also been inversely associated with liver fibrosis and degree of necroinflammation in NASH.[170] It is important to note, however, that differences in adiponectin levels between patients with simple steatosis and NASH are still not clear.[171-173] The impact of adiposity on adiponectin concentration should be considered in NAFLD. Adiponectin levels are inversely associated with truncal and abdominal fat, and positively associated with lower extremity fat. In fact, levels of adiponectin are markedly decreased in individuals with visceral obesity in particular and visceral obesity is a predictor of low of adiponectin.[136]

Leptin

Another adipokine, leptin, is mainly produced by adipocytes, though unlike adiponectin is considered to be a pro-inflammatory marker and important mediator of immune-responses.[163]

Animal and in-vitro studies identified the important role of leptin in appetite control and regulating body weight, as well as its antilipogenic effect on the liver.[163] These studies also identified that leptin can be deleterious in its role as a profibrogenic, pro-inflammatory and pro-diabetic cytokine.[171] Initial studies in obese humans showed that leptin deficiency improved glucose homeostasis, however leptin failed to correct hyperglycaemia introducing the notion of 'leptin resistance'.[136] In NAFLD patients, results are conflicting regarding leptin. Some studies have reported a positive association between increased leptin levels and degree of steatosis in NAFLD and NASH.[174, 175] While others – the majority of studies – have failed to show any association between leptin and steatosis or inflammation in NAFLD.[171, 176, 177] The role of leptin in NAFLD remains unclear and studies are required to clarify its role in the disease.

Resistin

Resistin is an adipokine, implicated in the regulation of inflammatory processes, and synthesised by adipocytes, muscle, pancreatic and mononuclear cells (such as macrophages) in humans.[163] In mouse models, resistin was identified to have a role in the pathogenesis of obesity-associated IR and T2DM,[136] however this finding has not been demonstrated in humans.[163] Resistin and adiponectin have opposing influences on systemic inflammation and effects on vascular endothelial cells differ; resistin promotes the expression of Vascular Cell Adhesion Molecule 1 (VCAM1), Intracellular Adhesion Molecule 1 (ICAM1) and pentraxin 3, whereas adiponectin downregulates the expression of these molecules.[178] One study reported elevated levels of resistin in a group of patients with NAFLD, when compared with obese or lean controls.[179] Human studies have not clearly identified the role of resistin in NAFLD, nor correlated IR with resistin levels. Aside from the aforementioned study, other studies report inconsistent results for resistin.[82] Other inflammatory markers, such as visfatin and CC-chemokine ligand 2 (CCL-2), have been reported to be higher in patients with NAFLD although additional studies are required to confirm these findings.[180, 181]

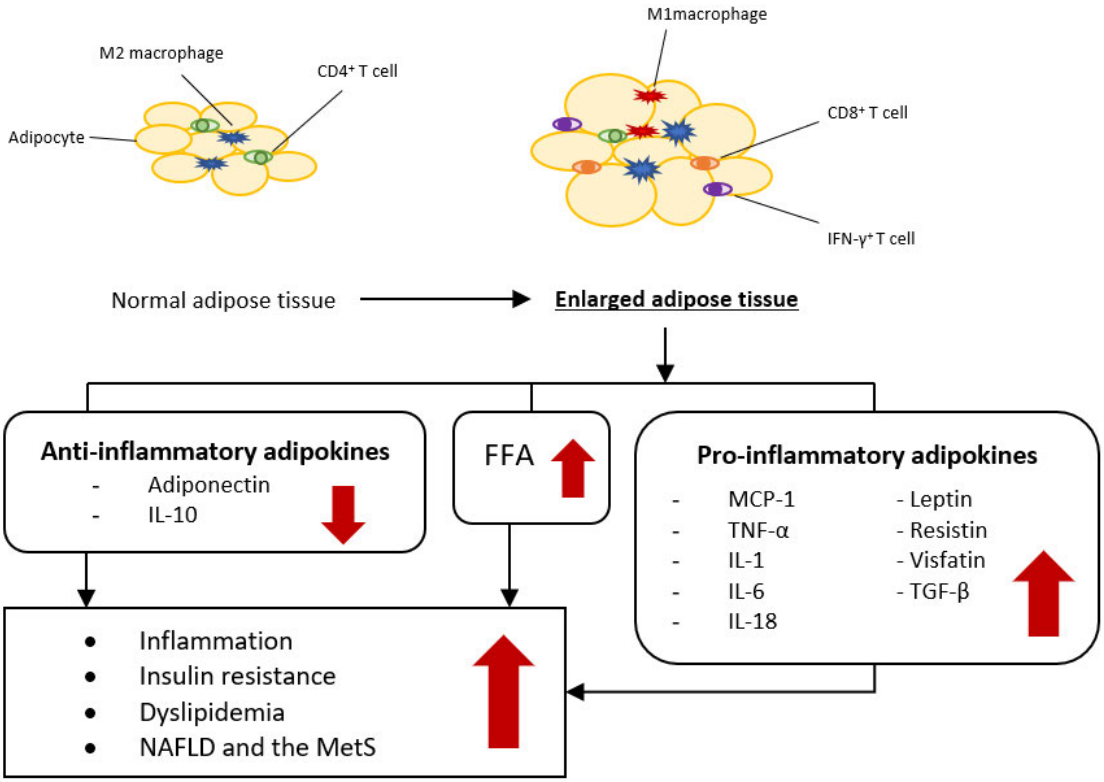


Figure 1.4. Secretion of inflammatory adipokines from enlarged adipose tissue in an obese state. *Adapted from Jung and Choi (2014).*[182] Enlarged adipose tissue leads to an increase in free fatty acids (FFA) and dysregulated synthesis of adipokines. Abbreviations: IL, interleukin; MCP-1, monocyte chemotactic protein; NAFLD, non-alcoholic fatty liver disease; MetS, Metabolic Syndrome; TGF-β, Transforming growth factor-β; TNF-α, tumor necrosis factor-α.

1.1.5 Genes influencing inflammation in NAFLD

Genetic factors are involved in an individual's susceptibility to developing progressive NASH. Genetic polymorphisms may influence the release or effect of cytokines and adipokines, although evidence of the functional significance of various gene variants is unknown and existing findings require replication.[183] Pro-inflammatory cytokine TNF-α is strongly implicated in mediating liver damage and TNF polymorphisms have been shown to influence susceptibility to NAFLD, as well as the transition to NASH.[184] Polymorphisms at positions -238 (called TNFA allele) and -308 (TNF2 allele) in the TNF promoter region have been extensively studied and whilst data is conflicting, most researchers accept that both polymorphisms are associated with increased susceptibility to NAFLD.[184] Wang et al. (2011) conducted a meta-analysis of 8 studies which investigated TNF-238 and -308; pooled data showed that prevalence of the -238 variant was higher in patients with NAFLD compared with healthy controls and associated with risk factors of NAFLD; IR and higher BMI, and the -308 variant led to elevated TNF-α production and was also associated with diabetes mellitus.[78] The specific effects and functional roles of TNF polymorphisms are still unclear.

IL-6 is a pleiotropic cytokine that plays an important role in many cellular and inflammatory processes, and mediates the balance between pro- and anti-inflammatory pathways.[184] In NAFLD and many other diseases, the presence of elevated IL-6 has been associated with the pathogenesis and/or progression of disease. The IL-6 -174 polymorphism may control the rate of transcription and resulting expression of circulating IL-6; however, some allelic differences have been reported. Two phenotypes for this polymorphism exist: G/G and G/C genotypes are characterised as 'high-producer' phenotypes wherein higher circulating IL-6 is present; and C/C genotype is the 'low'-producer' phenotype where lower concentrations of IL-6 are observed.[151] The IL-6 -174C variant was more prevalent in patients with NAFLD compared with healthy individuals, and was associated with insulin resistance and identified as an independent predictor of NAFLD and NASH.[185] Ethnic differences have been reported in population genetic studies of the IL-6 -174 variant. The aforementioned study was in Caucasian patients,[185] whereas other studies of Caucasian and Pima Indian patients with NAFLD have shown that the IL-6 -174G variant was associated with lipid abnormalities and prevalence of T2DM and in a Taiwanese cohort, authors concluded that the C allele was unlikely to play a role in the development of diabetes.[186, 187] A study of interleukin-10 deficient mice with ALD/NAFLD offers a possible explanation for these contradictory results. Following a high-fat diet, the mice had an increased liver inflammatory response with coinciding resistance to steatosis and hepatocellular damage due to an increase in hepatic IL-6/STAT3 activation, which consequently lowered lipogenic genes whilst up-regulating genes associated with FA oxidation in the liver.[188]

Adiponectin, an adipocyte derived hormone with anti-inflammatory, anti-diabetic and cardioprotective properties, is regulated via the adiponectin-encoding gene (ADIPOQ).[189] In patients with chronic disease including NAFLD, adiponectin is decreased. The most common SNPs in the exon/intron 2 of ADIPOQ gene (−11377C/G, +45T/G and +276G/T) have been associated with susceptibility to NAFLD.[190] Results of a study by Gupta and colleagues suggested that haplotypes −11377C/G and +45T/G may have functional significance in the pathogenesis and progression of NAFLD. The G allele of the −11377 variant was associated with higher necro-inflammatory grade, while the G allele of the +45 variant correlated with low serum adiponectin which may predispose hepatic steatosis.[191] In a meta-analysis of the three SNPs: −11377 G allele and +45 G allele were significantly associated with increased risk of CVD, whereas +276 T allele was associated with decreased risk.[190] The +276G/T polymorphism has been associated with decreased adiponectin expression, likely to be occurring alongside increases in body weight and/or visceral adipose tissue, IR and subsequent development of NAFLD.[192] A meta-analysis of 8 case-control studies involving 1639 NAFLD patients and 1426 controls indicated that adiponectin +275G/T was associated with NAFLD, and might be related to increased susceptibility to NAFLD.[192] Specific genotype effects of the +276 variant differ; GG homozygous carriers have been found to have lower circulating adiponectin and higher levels of IR compared to carriers of the T allele. These genotypes have also been associated with different responses to intervention, as GG

homozygote carriers experienced improvements in adiponectin concentration and reductions in IR following modest weight loss.[193] Numerous large, case-control studies of ADIPOQ gene variants are needed to replicate existing literature and to adequately determine genotype differences and susceptibility to NAFLD.

The acute-phase protein, CRP, widely used as a prognostic indicator of cardiovascular disease in patients with the MetS is a well-studied in the context of NAFLD. Evidence indicates that circulating CRP is higher in individuals with NAFLD or NASH, and increases with increasing grades or stage of disease.[194] The individual SNPs in the CRP gene are less studied in the context of NAFLD. Gene variant CRP +1846C/T may be an important influencer of circulating CRP, with one study reporting that carriers of the G allele had significantly higher levels of CRP increasing in a dose-dependent manner when compared with the AA genotype. The slope of increase in CRP was found to correlate with increase in BMI and waist circumference, suggesting that CRP gene expression may be mediated in part by adipose tissue.[195] Studies have identified several other genes which may influence the effect or release of cytokines/adipokines in NAFLD, namely: leptin receptor gene (LEPR) polymorphisms, Toll-like receptor-4 (TLR4) and Interleukin-10 (IL-10).[77, 183, 184] There is preliminary evidence to support the theory that gene variants have the potential to influence susceptibility to NAFLD, as well as disease pathogenesis and progression. However, larger well-documented studies exploring the mechanistic effect of SNPs are required to strengthen genetic associations described here.

1.1.6 Assessment and Diagnosis of NAFLD

Early detection and diagnosis of NAFLD is a crucial component in the management of silent simple steatosis and prevention of progression to NASH cirrhosis and liver-related comorbidities. Various clinical, biochemical and radiographic tests are widely accepted in the detection of NAFLD, though limitations in practicality, resources, expense and invasiveness exist in current tools. The three main features which are ultimately considered in the diagnosis of NAFLD across the disease spectrum include: histopathological features of liver damage and presence of intrahepatic fat, no indication of excess alcohol use as a cause of the disease, and a widespread investigation to ensure no other cause of chronic liver disease can be identified.[196]

1.1.6.1 Ultrasonography and Computed Tomography (CT)

The main forms of imaging used in clinical practice for detecting steatosis are ultrasonography and CT. These techniques are all widely accepted for their ability to detect moderate to severe steatosis, though are limited in the ability to differentiate between histological subtypes of simple steatosis, NASH and further stages of fibrosis. Abdominal ultrasound is commonly used for diagnosis of NAFLD in primary and secondary clinical care settings, benefits of this imaging include a non-invasive and inexpensive approach with low exposure to radiation. Sensitivity and specificity of ultrasonography has been reported as 60-90% and >90%, respectively, in patients who are not

obese. The main limitations of ultrasonography are that it is operator dependent which may result in inter- and intra-user variability, and in morbidly obese patients with <33% IHL content sensitivity of the test is limited.

Whilst CT can be useful for assessing hepatic steatosis in specific clinical cases, it has limited accuracy for detecting mild steatosis with the potential hazard of radiation exposure.[12] Decreased liver attenuation on CT typically suggests hepatic steatosis and the mean liver-to-spleen ratio has been validated with a sensitivity of >80%.

1.1.6.2 Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

Proton magnetic resonance spectroscopy (^{1}H-MRS) is the gold standard imaging technique used to detect NAFLD. This method is superior to others in quantifying IHL accurately and non-invasively, and has been proven to provide consistent quantitative measures of hepatic steatosis; sensitivity 88% and specificity 93%.[197, 198] The main limitation of ^{1}H-MRS is that it is expensive and not broadly available in clinical practice, therefore its current use is limited to research.[199]

1.1.6.3 Transient Elastography (TE)

Transient Elastography such as Fibroscan® is routinely used in the clinical setting as a rapid and non-invasive measure of liver stiffness. It is most effective at quantifying liver fibrosis using pulse-echo ultrasound, a technique equipped with a probe that transmits a vibration of mild amplitude and low frequency to the tissue inducing an elastic shear wave to propagate through the tissue. The pulse-echo ultrasonic acquisitions then measure the wave velocity, which is indicative of liver tissue stiffness. The faster the shear wave propagates, the harder the tissue.[200] Meta-analyses have suggested that liver stiffness measure (LSM) quantified from Fibroscan® is most reliable in assessing advanced liver fibrosis and early cirrhosis in chronic liver disease,[201] and while it may be useful, it is considered less accurate in NAFLD patients. Hepatic steatosis may decrease the elastic sheer wave passing through the tissue, though it does not affect the underlying speed used to measure LSM. Studies have shown a positive correlation between LSM and severity of fibrosis in NAFLD patients. Establishing stage of fibrosis in NAFLD patients is important in clinical perspectives because these patients are at high risk of developing rapid and asymptomatic complications. Fibroscan® is quick and easy to perform, relatively inexpensive and non-invasive, and results do not rely on subjective interpretation. However, a considerable limitation of measuring LSM in this group is the failure to obtain accurate results with increased BMI.

Controlled attenuation parameter (CAP) is a more recent imaging technique which uses a TE (Fibroscan®) probe and relies on the assumption that steatosis affects ultrasound propagation measures.[202] Initially, studies reported this technique to have good accuracy and sensitivity for imaging, even in patients with low levels of steatosis.[201, 203] Subsequent prospective studies,

however, have reported lower accuracy.[200] Therefore, validation of CAP is required in specific etiologic groups and histological features specific to NAFLD aetiology need to be considered.

1.1.6.4 Liver Biopsy

Liver biopsies remain the gold standard diagnostic tool for NAFLD as they provide a direct measure of disease severity.[204, 205] However, there are several limitations in performing this invasive, costly, labour intensive procedure on individuals presenting with elevated liver enzymes in the face of no proven pharmacotherapies. Liver biopsies are performed when there is a need to gather more comprehensive information of a patient presenting with some, if not all, of the following;

- elevated alanine aminotransferase (ALT) more than twice the normal concentration;
- elevated aspartate aminotransferase (AST) greater than ALT;
- moderate visceral adiposity;
- T2DM or impaired glucose tolerance;
- and hyperlipidaemia.[206, 207]

Various scales have been developed to describe the stages of liver damage from results of liver biopsies. Brunt et al. (1999)[205] developed a commonly used five-stage scale to encompass all grades of liver damage;

Stage 0 – absence of fibrosis;
Stage 1 – perisinusoidal or portal fibrosis;
Stage 2 – perisinusoidal and portal/periportal fibrosis;
Stage 3 – septal or bridging fibrosis;
Stage 4 – cirrhosis.[208]

The NAFLD activity score (NAS) represents features of active liver injury and is used to grade reversible injury. The score is comprised of steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2), and the unweighted sum (0-8) is used to grade activity. The NAS was developed for its use in intervention studies to demonstrate changes in histology before and after therapy.[109] Fibrosis is separated from the NAS score because it is considered to be less reversible and dependant on disease activity.[209]

Histopathological presentation of NAFLD resembles alcoholic fatty liver disease (ALD), and is only differentiated by rigorous assessment and exclusion of alcohol consumption as the cause of liver damage.[210] Although liver biopsy can provide comprehensive clinical assessment of the state of the liver, diagnosis of NAFLD versus ALD is not possible from histopathological examination alone.[210] Besides excess alcohol consumption, NAFLD is distinguished from ALD by serum ALT/AST ratio, whereby; ALT is greater than AST in NAFLD, and the reverse is most often

observed in ALD. Use of liver enzymes to diagnose and/or differentiate between liver disease is limited, as NAFLD progresses to NASH (with fibrosis) ALT levels may drop causing the offset of ratios. Serum AST has also been poorly correlated with histological activity. Liver enzymes should be interpreted with care and not used to guide diagnosis of significant liver disease. Liver biopsy is the only diagnostic procedure that can identify steatosis from steatohepatitis, while also classifying the grade/severity of liver disease.[210]

The histological stage of NAFLD at time of presentation will determine the prognosis of disease. Clinical evidence indicates that 1-2% of patients presenting with simple steatosis have moderately benign liver disease and are likely to develop cirrhosis over 15-20 years.[204] Alternatively, patients who present with NASH and fibrosis are at increased risk (~12%) of developing cirrhosis within 8-years.[204]

1.1.6.5 Common Biomarkers for the Diagnosis of NAFLD

Traditionally, elevated liver enzymes were the first indication of abnormalities in liver function and further investigation often resulted in subsequent diagnosis of NAFLD. Mildly elevated serum ALT is the first abnormality observed in patients with NAFLD indicating increased risk of liver dysfunction. However, substantial evidence has shown that up to 78% of patients with NAFLD may display normal liver enzymes and entire histological presentation of NAFLD has been observed in some patients with normal ALT values.[211, 212] A diagnosis of minimal liver fibrosis has been associated with AST/ALT ratios of less than 1, while a ratio greater than 1 has been associated with development of cirrhosis.[7] No single biomarker can confirm diagnosis of NAFLD, nor distinguish between steatosis, NASH and/or fibrosis.[212] Serum gamma-glutamyl transferase (GGT) is often elevated in patients with NAFLD, though it is not solely used for diagnosis of NAFLD.[10] Increased levels of GGT have been associated with advanced fibrosis, specifically, in a group of 50 patients with NAFLD a cut-off value of 96.5U/L predicted advanced fibrosis; 83% sensitivity and 69% specificity.[213] Alkaline phosphatase (ALP) is rarely used to assess risk or development of NAFLD, it is sometimes slightly elevated in NAFLD patients.[214] Up to 50% of patients with NASH have elevated levels of ferritin and approximately 10% have elevated transferrin, however the role of ferritin, transferrin and hepatic iron in the pathogenesis of NASH is uncertain.[7]

The need for novel biomarkers to support the diagnosis of NAFLD is acknowledged by investigators given the low predictive value of non-invasive tests, the lack of current measures' capacity to distinguish between simple steatosis and more advanced inflammation or fibrosis, the high cost of testing and the risks associated with liver biopsy.[10, 46] Ideally, novel biomarkers will provide a screening and monitoring tool for NAFLD, as well as a predictor of disease progression and prognosis.

1.1.6.6 Inflammatory Markers in NAFLD

Chronic low-grade inflammation has been identified as an underlying feature of metabolic disorders, and has increasingly been associated with the development of hepatic steatosis and progression to NASH.[10] Investigators have proposed that markers of inflammation may be used as potential diagnostic tools, though the current evidence for inflammatory markers is limited by lack of reproducibility and specificity in hepatic outcomes, as well as the unclear role of cytokines and adipokines in advancing liver damage, and consistent response to therapeutic interventions.[10]

Of the inflammatory markers (cytokines and adipokines) introduced earlier; CRP lacks specificity for hepatic outcomes and clinical and prognostic implications are unclear,[155] the extent to which adiponectin, TNF-α and IL-6 are markers of hepatic fibrosis and liver injury and not a direct effect of more severe insulin resistance or increased adiposity is yet to be established,[82] and the degree to which leptin and resistin correlate with steatosis or fibrosis has not yet been identified.[10, 177] Evidence for the use of inflammatory markers in monitoring the progression of NAFLD over time, it's response to dietary or other therapeutic interventions and determining prognosis of the disease is required prior to using a novel inflammatory biomarker for diagnostic purposes.

1.1.6.7 Non-invasive Scoring Systems

There are various scores that have been developed to detect and/or predict NAFLD which utilise non-invasive markers such as biomarkers, anthropometry and demographics. Briefly, the Fatty Liver Index (FLI) score was derived from the population of the Dionysos Nutrition & Liver Study and incorporates BMI, waist circumference, triglycerides and GGT, to calculate a score between 0 and 100.[215] The score, derived from routine clinical measurements, was designed to assist the selection of patients to be referred for ultrasonography and to identify patients in need of more intensified therapeutic treatment.[215] The accuracy of detecting fatty liver is 0.84 (95% CI 0.81–0.87) and the FLI has been widely used in epidemiological studies, where ultrasound or imaging data is not available in large population datasets.[215-217] The same research group also developed a simpler measure of steatosis, the Lipid Accumulation Product (LAP), which is calculated using waist circumference and triglycerides. The LAP is reasonably accurate (accuracy of 0.8), though requires validation in independent groups.[218]

Kotronen et al. (2009) developed the NAFLD Liver Fat Score which was derived from a Finnish population includes the presence of the MetS and T2DM as variables, as well as fasting insulin, AST and AST/ALT ratio. This score predicted increased liver fat content with sensitivity of 86% and specificity of 71%.[219] Both of these scores were intended to be simple, reasonably accurate non-invasive predictors of liver steatosis and although they have been tested against ultrasonographic methods, they do not include any liver tissue tests (ultrasound, biopsy or other). These scores are commonly used in research to select and monitor patients for epidemiological studies.[218] Other

scoring systems have been developed for the staging of NAFLD fibrosis, however these scores will not be reviewed for the purpose of this thesis.

1.1.7 Management Strategies

The current management strategies for individuals diagnosed with NAFLD are targeted at lowering metabolic risk factors, comorbidities and treating liver disease. Individuals who are at-risk of developing NASH should be referred to appropriate health clinicians for comprehensive assessment and optimal management, often delivered in collaboration with a multidisciplinary team to manage treatment strategies. Current interventions aimed at reducing risk of disease progression in individuals presenting with NAFLD fall into three categories: surgical, pharmacotherapy and lifestyle.

1.1.7.1 Surgical

In obese (or morbidly obese) patients who do not respond to lifestyle changes or pharmacotherapy, bariatric surgery is one strategy that may reduce weight and metabolic complications.[46, 220] Bariatric surgery is rarely indicated as the primary option for hepatic disease and the effect of bariatric surgery on NAFLD-associated liver injury and associated metabolic risk factors are not fully known. Three published meta-analysis' have investigated the effects of bariatric surgery on weight and metabolic risk in patients with NAFLD, though results are inconsistent.[221-223] Mummadi et al. (2008) extracted data from 15 studies and total of 766 paired liver biopsies, which showed the pooled proportion of patients with complete resolution in histopathologic NASH after surgery was 69.5% (95% CI, 42.4%–90.8%).[222] Similarly, Fakhry et al. (2008) reported an 88% post-operative improvement of steatosis (95% CI, 0.80-0.94) and a 30% improvement or resolving of fibrosis (95% CI, 0.21–0.41) in 2,374 patients with NAFLD across 21 studies.[224] More recently, a comprehensive review of 32 studies comprising of 3,093 biopsy specimens reported biopsy-proven resolution of steatosis in 66% of patients (95% CI, 56%–75%) and resolution of fibrosis in 40% (95% CI, 29%–51%).[223] However, histologic worsening or development of new features of NAFLD (such as fibrosis) was reported in 12% of patients (95% CI, 5%–20%).[223] Currently, there is not substantial evidence to recommend bariatric surgery as a first-line therapy for NAFLD.

1.1.7.2 Pharmacotherapy and Supplementation

Although clinical trials are ongoing, there is no approved safe, effective pharmacotherapy for NAFLD or NASH. High-quality evidence using randomised controlled trial study designs, that are powered to determine change in liver histology and can establish long-term efficacy or safety of the pharmacologic agent, are few.

Briefly, current recommended pharmacotherapies for NASH/NAFLD in guidelines[46, 225, 226] or guidance[35] include vitamin E, pioglitazone, metformin, glucagon-like peptide-1 receptor agonists (GLP-1RAs) or statins.[227] Vitamin E has been proposed in the treatment of NASH, as it may reduce

oxidative stress and improve serum transaminase activities and insulin resistance.[227] Although some clinical trials have reported superior effects for vitamin E on NASH histology,[228, 229] vitamin E treatment may cause toxicity and increase all-cause mortality,[230] prostate cancer[231] and haemorrhagic stroke[232] with long-term or high-dose use.[227] Treatment of NASH with vitamin E should be considered in lower-doses and monitored. Anti-diabetic/insulin-sensitizing and lipid-altering agents have also been explored for treatment of NAFLD/NASH. The key problem in administering drugs such as pioglitazone, metformin, GLP-1RA (anti-diabetic/insulin-sensitizing medications) or statins (lipid-lowering agents) is lack of data regarding improvement in liver enzymes and histology in patients with NAFLD or NASH.[227] Moreover, prospective randomised controlled trials (RCTs) are difficult to perform due to conflicting preliminary results from pilot drug studies.[227] Ideally, pharmacological treatments for NASH should have weight-reducing efficacy, lower risk of CVD-events, prevent HCC, as well as being cost effective and improve QOL.[233] The most significant question posed by investigators is at which point in NAFLD/NASH pharmacologic treatment is warranted. A recent meta-analysis of five adult NAFLD cohort studies determined that the presence of advanced fibrosis (≥stage 2) was the most important predictor of liver-related mortality in NAFLD patients.[234] Guidelines from the American Association for the Study of Liver Diseases suggest that pharmacotherapies should be limited to patients with NASH and fibrosis,[35] and patients with non-aggressive type of NAFLD or NASH (stage 0) do not require liver specific treatments.

Promising supplements such as omega-3 (n-3) fatty acids (FAs), vitamin D and probiotics have been increasingly investigated as therapeutic agents for the treatment of NAFLD.[227] n-3 FAs bear lipid-lowering and anti-inflammatory effects, and a recent systematic review and meta-analysis of 22 RCTs found that n-3 PUFA supplementation also significantly reduced total triglyceride, total cholesterol, HDL-c and BMI compared to placebo.[235] Authors of this review established, however, that many of the included RCTs were high in risk of bias and contained small sample sizes. Moreover, there were no histological improvements observed in the studies and dose-dependency remained unknown.[235] A recent systematic review and meta-analysis of ten trials with a total of 544 NAFLD participants assessed vitamin D supplementation on liver enzymes and cardiometabolic risk factors.[236] The study found that whilst supplemental vitamin D was effective in improving glycaemic control (fasting glucose, insulin, and HOMA-IR), there was no significant effect of vitamin D on liver enzymes or lipid profiles.[236] A systematic review of 28 clinical trials assessed the use of probiotics in NAFLD and found that overall, probiotic therapy had beneficial effects of BMI, liver enzymes, HOMA-IR and total cholesterol, but not fasting glucose, lipid profiles or TNF-α.[237] Similar to the aforementioned supplementation studies, the results for probiotic treatment were ascertained from small sample sizes over a short duration of intervention (≤6-months).[237] Higher quality, long-term RCTs are warranted to investigate liver steatosis, indexes of liver fibrosis and inflammatory markers, to establish a comprehensive evidence-base for the efficacy of n-3 FA,

vitamin D and probiotic supplements in the treatment of NAFLD. This evidence will further inform the development of relative practice guidelines.

1.1.7.3 Lifestyle Intervention and Weight Loss

Lifestyle modification remains the first-line therapy for patients with NAFLD. Dietary intervention resulting in weight loss can improve hepatic steatosis, liver enzymes and IR in patients with NAFLD.[238, 239] In a systematic review of 24 studies, researchers assessed the efficacy of diet and physical activity (PA) interventions in NAFLD, finding that while all studies resulted in significant reductions in steatosis and/or markers of NAFLD activity, combination (diet and PA) interventions were most effective at improving NAFLD.[240] Evidence from the intervention studies indicated that weight loss of 5% total body weight improved hepatic steatosis, whereas 7-10% lead to significant improvements in histological NASH (NAS activity score).[240] Studies that achieved a greater amount of weight loss used hypocaloric or very-low calorie diets (VLCDs),[240] and it is known that a dose-response curve exists wherein the greater amount of energy restriction and weight loss, the more significant improvement in histopathology of all features of NASH.[35] Another systematic review which included eight RCTs investigated the effects of diet and lifestyle interventions on IR in patients with NAFLD. Authors found that intervention groups which modified diet alone or included diet and PA were effective in reducing HOMA-IR scores by up to two units but did not achieve significant weight loss in patients with NAFLD, whereas lifestyle interventions which incorporated an energy-restricted diet most often resulted in weight loss and a reduction in HOMA-IR in overweight and obese patients with NAFLD.[238] From this review it remained unclear which diet and lifestyle intervention was most effective in lowering IR in patients with NAFLD. However, authors conclude with a more important aim for future studies, in that the optimal composition of diet or diet and PA models for achieving metabolic health benefits and physiological effects of hypocaloric diets in this population are yet to be established. [238]

Diets which include a calorie-deficit (or energy-restriction) to induce weight loss have been investigated as "intervention diets" in NAFLD populations, in that the duration of an intervention study is typically no longer than 6-months. Data for the sustainability of energy-restricted or hypocaloric diets, as well as the weight loss that they induce, is not widely reported in NAFLD. One prospective study in patients with NAFLD reported that only 50% of patients were able to achieve 7% weight loss by 12-months.[241] It is a difficult and often unattainable task for patients to adhere to a calorie restricted regimen (with or without PA) and sustain weight loss in the long term,[242] and loss to follow-up or missed appointments is frequent in the clinical setting.[47] Therefore, lifestyle interventions should also aim to provide hepatic and cardiometabolic benefits, independent of weight loss. Traditionally, the specific macronutrient composition of diet was considered less relevant than the result of diet-inducted weight loss. However, in more recent years it is understood that in order to improve sustained weight loss through diet and lifestyle interventions, the specific role of nutrition (macro- and micronutrients along with type of diet and calories) on clinical

outcomes needs to be considered. While the optimal dietary pattern for management of NAFLD has not yet been defined, key guidelines such as the EASL-EASD-EASO Clinical Practice Guidelines for the Management of NAFLD acknowledge that the Mediterranean Diet has had superior effects on liver fat when compared to a low-fat/high-carbohydrate diet.[46] These guidelines recommend that macronutrient composition should be adjusted according to the Mediterranean diet.[46] Indeed, additional research investigating diet and lifestyle interventions such as the Mediterranean Diet are needed to support long term weight reduction and maintenance, whilst efficiently lowering hepatic steatosis, inflammation and mediating metabolic processes without reliance on weight loss.[243] Well-powered, rigorous, long-term prospective studies with primary histopathologic endpoints are required to document and compare diets with differing macronutrient compositions.

While there are no specific PA recommendations for patients with NAFLD, an active lifestyle is encouraged by guidelines and practitioners.[35] In the aforementioned systematic review, combination interventions (diet and PA) were more effective in improving NAFLD and achieving weight loss, compared to diet alone.[240] Most of the protocols involved moderate-intensity exercise for 30-60 min on 3-5 days per week.[240] Benefits have been described for both resistance and aerobic exercise for NAFLD, there is no consensus concerning type of exercise in the current guidelines.[220] Ultimately, avoiding a sedentary lifestyle is important in limiting the accumulation of adipose tissue and metabolic disturbances that may impact liver health.[244]

1.2 Habitual Dietary Intakes in NAFLD

Dietary intake of individuals who develop NAFLD is characteristically high in saturated fat, cholesterol and refined carbohydrates.[245] Highly processed food and beverage items, usually high in added sugar, contribute to excess total energy and fructose intake in this population.[246] Generally, studies report lower intakes of fruits and vegetables, dietary fibre and antioxidants and omega-3 in individuals with NAFLD.[245] These diet characteristics are implicated in the development of metabolic abnormalities and chronic disease, and not only promote the improper storage of fat in adipose tissue but also aid inappropriate release of fatty acids into circulation leading to hepatic steatosis and activation of inflammatory pathways. Epidemiological studies have reported that consumption of unhealthy diet patterns and adoption of a sedentary lifestyle significantly increase the risk of developing NAFLD, the severity of the disease and its progression to NASH.[27] In particular, globalization of a 'Western Diet' characteristically higher in red and processed meat, sweets and desserts, fried food, and refined grains is associated with an increase in the prevalence of NAFLD in developing nations, a trend that is increasingly likely to be observed in Asia in the coming years.[247] There is a positive correlation between the Western dietary pattern and level of CRP.[247] Moreover, a Western Diet increases chronic underlying inflammation and raises the risk of developing T2DM, NAFLD and CVD.[248, 249]

1.2.1 Dietary Approaches to the Management of NAFLD

Previous literature has focused on finding associations between the consumption of specific nutrients and/or reliance on certain food groups with detrimental effects to liver health, and subsequent clinical trials largely aimed to understand the effects of these isolated components. This data assists researchers and clinicians to understand the complexity of the pathogenesis of NAFLD and the need to assess dietary intakes and to understand the mechanisms of macro- and micronutrients in the development and progression of hepatic steatosis. However, this method does not provide a holistic dietary approach for the prevention and treatment of NAFLD.[250, 251] In more recent observational cohort and RCTs, the role of whole of diet approaches and dietary patterns are increasingly considered in improving clinical characteristics of NAFLD.[4, 38, 252] Since NAFLD is a multifactorial disease, the complex and synergistic effects of nutrient interactions may be important for the improvement of not one, but many NAFLD related outcomes.

1.2.1.1 Healthy Dietary Patterns

Various 'healthy' dietary guidelines are recommended in the context of managing NAFLD, including; the American Heart Association (AHA) guidelines,[253] the National Cholesterol Education Program (NCEP)[254] and the Dietary Approaches to Stop Hypertension (DASH).[255] Dietary intervention studies supporting the usefulness of these guidelines for outcomes of hepatic steatosis and inflammation in NAFLD cohorts are reported in a systematic literature review published and embedded in this thesis by the doctoral candidate [256] (**Section 1.2.5**).

Briefly, the AHA typically focuses on three aspects or risk factors of heart disease; high cholesterol intake, high blood pressure and excess body weight.[253] The basic recommendations set by the AHA are to consume fish twice a week, avoid trans-fatty acids, limit intake of saturated fatty acid (SFA) to <10% total energy (or calories) and limit cholesterol intake to <300mg/d. As part of a joint statement for the Diagnosis and Management of the Metabolic Syndrome the AHA and the National Heart Lung and Blood Institute (NHLBI) further advise maintaining a total fat intake of 25-35% total energy (or calories) while further decreasing SFA to <7% total energy (calories), decreasing intake of simple sugars and increasing intakes of fruits and vegetables.[103] Recommendations set by the AHA target dietary patterns known to be problematic for people who develop NAFLD and are therefore suitable for those with NAFLD.

The NCEP Adult Treatment Panel III or Therapeutic Lifestyle Change diet was developed and modified to address the increasing incidence of CHD and targeted at individuals considered "high-risk".[254] The NCEP dietary guidelines recommend total fat intake of 25-35% of energy intake (<7% SFA, up to 10% PUFA and up to 20% MUFA), carbohydrate intake of 50-60% of energy intake and protein intake ~15% of total energy.[254] It is also recommended that cholesterol intake be limited to <200mg per day, sodium intake to <2400mg per day and intake of fibre up to 20-30g per day. These dietary changes, with an increase in physical activity, are expected to lead to weight reduction

and maintenance of a healthy weight.[254] Few studies have investigated the effects of an NCEP Adult Treatment Panel III therapeutic lifestyle-change diet in NAFLD, and the two studies that have, also supplemented diet groups with either L -carnitine[257] or *Bifidobacterium longum* with fructo-oligosaccharides (Fos)[258] versus placebo. The first study found that diet alone and diet with L-carnitine decreased NASH-activity score by at least two points, though L-carnitine had a significantly greater effect than placebo.[257]. Similarly, the NCEP diet alone or with supplementation of *Bifidobacterium longum* with fructo-oligosaccharides (Fos) resulted in significantly reduced NASH activity score in both groups, and again the effect was significantly greater with supplementation compared to placebo.[258] The NCEP/Therapeutic Lifestyle Change diet guidelines may be beneficial for management of NAFLD, though longer term diet-only studies are required to support initial findings for hepatic and inflammatory outcomes.

The DASH eating plan was created for the purpose of reducing blood pressure in the management of hyptertension;[259, 260] however, its beneficial effects on metabolic profiles, biomarkers of inflammation and oxidative stress have also been reported in T2DM.[261, 262] The basic principles of the DASH diet follow the United States Department of Agriculture (USDA) Dietary Guidelines, which focus on consuming a wide variety of fruit, vegetables and wholegrain products and low intake of "discretionary" products.[260] The USDA recommendations are customised to body size and activity level but do not address weight reduction or the needs of individuals with metabolic abnormalities..[260] Recently, large epidemiological studies have shown that higher DASH scores were inversely associated with NAFLD risk and liver fat content.[263-265] Intervention studies exploring the efficacy of the DASH diet for management of NAFLD are lacking. One RCT has tested the effects of a DASH diet versus energy-restricted diet in patients with NAFLD, reporting significant improvements in body weight, liver enzymes, insulin sensitivity, lipid profile, inflammatory markers and oxidative stress in the DASH diet group compared to the energy-restricted diet.[255] Short-term, controlled studies have reported the effectiveness of the DASH diet in other outcomes, such as lowering hypertension, however long-term and/or real-world studies are lacking due to the restrictiveness and low palatability of the diet.[260, 266] Associations between the DASH diet pattern and NAFLD are promising, however intervention studies are warranted for it to be considered a reasonable approach in the management of NAFLD.

Low-carbohydrate diets (~<40% total energy from carbohydrate) promote rapid weight loss along with improvements in IR and associated MetS components, and are therefore considered a potential treatment option for NAFLD.[267] A meta-analysis of four studies found that low-carbohydrate diets (<50% total energy from carbohydrate) significantly decreased liver fat content in 50 adults with NAFLD.[268] While studies have shown effective short-term effects of a low-carbohydrate diet, the lack of impactful long-term studies and high drop-out rates in published RCTs indicate that adherence to these diets may be difficult and may actually promote the re-development of metabolic outcomes experienced in NAFLD.[267] Alternatively, moderate carbohydrate diets may be more

palatable and are reported to lower liver enzymes in individuals at risk of NAFLD, whilst improving liver histology in individuals diagnosed with NAFLD.[269, 270] A study of 68 outpatients who followed either a low carbohydrate or moderate carbohydrate hypocaloric diet for 12-weeks reported weight and adipose tissue loss was similar between the two groups, and fasting blood glucose, cholesterol and triglycerides significantly decreased almost identically in both groups.[271] Fasting insulin decreased to a greater extent in the low carbohydrate diet compared to moderate carbohydrate diet.[271] Based on the findings of these studies moderate carbohydrate restriction (~40-50% total energy from carbohydrate) may be a reasonable option for NAFLD patients to sustain.

1.2.2 The Low-Fat Diet

The Low-Fat Diet (LFD) is recommended in the current Australian Dietary Guidelines and Australian National Heart Foundation guidelines.[101, 272] The LFD is recommended to patients presenting in an Australian clinical setting for management of NAFLD, though there remains a paucity of evidence for the diet in NAFLD patients.

Restriction of dietary fat was routinely recommended for patients diagnosed with NAFLD, as the amount of dietary fat consumed was thought to be directly proportionate to IHL accumulation.[46] Diet can significantly alter liver composition and similar to low-carbohydrate diets, low-fat diets tend to induce weight loss due to the restriction of energy. A study of 170 overweight and obese individuals randomised to either a low-fat or low-carbohydrate diet indicated that both diets decreased IHL content, total body and visceral fat and body weight.[273] There was also a reduction in insulin resistance following both diets. Observed changes were irrespective of dietary composition and appeared to be independent of visceral fat or insulin change.[273] In a similar study by De Luis et al. (2010),[274] 162 obese individuals were recruited, of whom 28 had NAFLD, and placed on a LFD or low-carbohydrate diet for 3-months. Researchers found that both diets induced similar amount of weight-loss (4-5%) and decreased IR to a similar degree. Furthermore, weight-loss was associated with a reduction of anthropometric measures, fasting glucose, total cholesterol, LDL-c and IR..[274] Within the clinical setting, low-fat dietary intervention remains the mainstay treatment for NAFLD, though the confounding influence of weight-loss means that the effects or benefits of nutrient composition on drivers of pathogenesis − such as inflammation − remain relatively unexplored.[275]

1.2.3 The Mediterranean Diet

Recently, the Mediterranean Diet (MedDiet) was recommended as the optimal dietary pattern in the management of NAFLD by the EASL-EASD-EASO Clinical Practice Guidelines.[46] The MedDiet is increasingly studied and reported in the literature across a number of countries, ethnicities and diseases or comorbidities. Whilst adherence to MedDiet has been associated with lower rates of NAFLD and improved hepatic and metabolic outcomes,[276] data supporting the MedDiet in NAFLD

from non-Mediterranean countries is still needed.[277, 278] Despite this, it is important to consider the basis of the traditional diet and its key components in order to fully understand its health benefits and functional effects.

The MedDiet was first described in the late 1950's by Keys and colleagues,[279] who identified the cardioprotective effect of traditional dietary patterns across 'Seven Countries' that surround the Mediterranean Sea; Spain, Italy, Greece, Turkey, North Africa, Lebanon and Israel. This study found that the incidence of coronary heart disease (CHD) was particularly low in Greek Island of Crete, owing to low content of saturated fat in the MedDiet and high consumption of plant foods and olive oil abundant in unsaturated fats.[279] Thus, the Cretan Diet is considered a 'traditional' Mediterranean dietary pattern which Keys himself described as a diet rich in whole grains, fruit, vegetables, and low in meat and processed foods, with a considerable amount of fat deriving from olive oil and nuts. This dietary pattern seemed to be a possible determinant of the wide difference in CVD prevalence between Mediterranean cohorts and the Western population in the Seven Countries Study.[279]

Since the Seven Countries Study, many cohort studies and clinical trials have continued to explore the beneficial effects of the MedDiet and its key diet components.[280-282] The MedDiet is largely plant-based and emphasises consumption of wholegrains and legumes, vegetables (particularly wild leafy greens, tomatoes, onions, garlic and herbs), fruits and nuts.[283, 284] The main culinary fat is extra virgin olive oil (EVOO), with little to no consumption of butter or margarine products. Protein from animal sources is advised through increased intake of fish and seafood, and moderate intake of poultry and eggs.[283, 284] Natural dairy products, mostly in fermented forms of yoghurt and cheese (feta), are consumed in moderate to low amounts along with minimal consumption of red meat. Processed foods and beverages are typically not consumed, and desserts or baked goods should be mostly homemade, consumed in small amounts and limited to special occasions. One serve of nuts or dried fruit (approximately 30 grams) may be consumed daily, as the most frequently chosen dessert. Wine (red wine) is allowed in moderation of one glass per day to be consumed with a meal. The MedDiet is characterised by a high ratio of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) and although it is a high-fat diet (35-45% energy derived from fats), contains specific dietary components which reduce inflammation and oxidative stress, and may alter body composition in the absence of weight loss.[283, 284] Namely, MUFA, omega-3 polyunsaturated fatty acids (PUFA), fibre, polyphenols, antioxidants including Vitamin E and C, and minerals such as zinc and selenium are nutrients and bioactive compounds contributing to the diets anti-inflammatory and anti-oxidant potential.[285] Traditionally, cultural identity, hospitality and cooking methods were key components in the MedDiet pattern. Cooking methods have been identified as an important element of the diet, which enhance the synergistic effects and nutritional benefits of individual food components.[281] A good example of this is the sofrito method; cooking of EVOO

with onion, garlic and tomatoes over low-medium heat, which allows for considerable increases in the absorption of lycopene (a carotenoid with anti-oxidant properties).[286]

In recent years an increasing number of studies have been published showing the beneficial effects of the MedDiet for treatment of hepatic steatosis in NAFLD.[46] A few observational studies and clinical trials have provided evidence for the MedDiet as a therapy for NAFLD. In Spain, a prospective cohort study placed 14 obese men with ultrasound-proven NAFLD on a "Spanish Ketogenic Mediterranean Diet" which resulted in complete fatty liver regression (21.4% of the patients), an overall reduction in 92.86% of patients (p <0.001) and significant BMI, AST and ALT reductions (p <0.001) after 12-weeks.[287] In Italy, a prospective study by Trovato et al.[278] placed a larger emphasis on cognitive-behavioural strategies and nutritional counselling rather than the composition of diet, and found that by using these strategies to encourage greater Adherence to the MedDiet Score (AMDS), significant decreases in hepatic fat (bright liver score), BMI and HOMA-IR (p <0.001) were observed. Meanwhile, another prospective study in Italy which administered a calorie-restricted MedDiet to 46 adults with ultrasound-proven steatosis showed that diet was effective in reducing the 52% of patients with severe steatosis to only 9%, with a remission of steatosis in 20% of the entire cohort.[288] Furthermore, fatty liver index decreased significantly (p <0.01), as did liver enzymes ALT, AST and GGT.[288] This study also highlighted the benefit of nutritional counselling which was reported to increase adherence to the diet and lifestyle regimen.[288] The impact of adherence to the MedDiet on severity of NAFLD was explored in a case-control study which found that the MedDiet score was inversely correlated with serum ALT (p =0.03), insulin levels (p =0.001), HOMA-IR (p =0.005) and severity of steatosis (p =0.006) and positively associated with serum adiponectin concentration (p =0.04).[289] Patients with NASH had significantly lower MedDiet adherence scores (p =0.004) than patients with simple fatty liver, and regression analyses revealed that a one unit increase in the MedDiet score would predict a 36% reduction in the likelihood of having NASH (OR: 0.64, 95% CI: 0.45-0.92), after controlling for sex and visceral adipose tissue.[289]

In Italy, researchers randomised patients with ultrasound-proven NAFLD to either; a low-calorie MedDiet, a low-calorie MedDiet with anti-oxidant supplements or a control which was the participant's usual diet (i.e., no treatment or intervention).[290] At 6-months, significant reductions in hepatic fat, (p =0.0001), fatty liver index (p <0.01), BMI (p =0.0001) and lipid profile (p <0.001) were reported in both MedDiet groups but not in the control group.[290] A variation of the MedDiet was investigated in another Italian study of NAFLD patients and it was found that a Low-Glycaemic Index MedDiet, not energy restricted, was effective in reducing fatty liver index and ALT (p <0.05).[291] In Greece, a randomised controlled single-blind clinical trial was conducted in overweight/obese patients with ultrasound-proven NAFLD. Participants were randomised to either a MedDiet, Mediterranean Lifestyle (ML; MedDiet with physical activity, optimal sleep and mid-day rest recommendations) or control group and followed up for 6-months.[292] Following

intervention, greater reductions in BMI (p =0.008) and liver stiffness (p =0.02) were observed in MedDiet and ML groups compared to control.[292] Most recently, a large clinical trial involving 278 participants with abdominal obesity, dyslipidemia and NAFLD diagnosed by MRI compared a low-fat diet to a Mediterranean, Low-Carbohydrate (MD/LC + 28 g walnuts/day) diet with or without moderate physical activity (PA).[293] Consequent decreases in hepatic fat content were similar across low-fat and MD/LC groups who undertook PA, however after controlling for changes in visceral adipose tissue the MD/LC group had greater reductions in hepatic fat content (p =0.036) and greater improvements in cardiometabolic risk markers (p <0.05). Further analyses showed that after controlling for visceral adipose tissue loss, reductions in hepatic fat content were independently associated with reductions in liver enzymes and glycated haemoglobin.[293] Additionally, an 18-month lifestyle intervention of 278 participants with abdominal obesity/dyslipidemia in Israel tested the effects of a low-carbohydrate MedDiet compared to a LFD, and found that the low-carbohydrate MedDiet significantly reduced hepatic fat content to a greater extent than the LFD, and this change was independent of VAT reduction.[294] The MedDiet was also associated with greater reductions in cardiometabolic risk markers than the LFD, though the difference was non-significant when controlling for the change in hepatic fat and remained significant after controlling for VAT and weight loss.[294] Thus, a low-carbohydrate MedDiet may elicit beneficial effects on cardiometabolic risk markers via decreases in hepatic fat content, rather than solely being mediated by loss of adipose tissue.

A few small-scale clinical trials have investigated the MedDiet in non-Mediterranean populations, including Australia, with encouraging results. In 2007, Ryan et al.[270] randomised 52 obese and insulin-resistant individuals to a hypocaloric low-fat, high carbohydrate diet or a moderate-fat, moderate carbohydrate diet for 16-weeks, found that both diets resulted in significant reduction of body weight, steady-state plasma glucose, circulating insulin and ALT. In 2013, Ryan et al.[295] conducted a 6-week cross over study in twelve non-diabetic patients with biopsy-proven NAFLD who followed either a Mediterranean diet (moderate-fat, moderate carbohydrate) or a low-fat, high carbohydrate diet. Following dietary intervention, researchers found that although weight loss did not differ between the two groups, hepatic steatosis and insulin sensitivity both improved in the Mediterranean diet compared with the low-fat, high carbohydrate diet.[295] More recently, researchers in Western Australia conducted an RCT in 48 patients with NAFLD to investigate the effects of a LFD versus MedDiet on hepatic steatosis and CVD-risk factors.[275] Properzi et al. (2018)[275] found that both diets reduced hepatic steatosis, liver enzymes and body weight to a similar degree, while dietary adherence was higher in the MedDiet group compared to the low-fat group.[275] In comparison to Ryan et al. (2013) who conducted a tightly-controlled trial including the full provision of meals over 6-weeks,[295] Properzi et al. (2018) administered an *ad libitum* dietary intervention over a duration of 12-weeks which may be a key reason for the differences in outcomes previously outlined.[275] Beneficial effects of the MedDiet in improving hepatic fat content, metabolic parameters and adiposity have been noted through various international and a few Australian

studies, though further investigation is needed to elucidate the mechanisms through which diet improves the 'multi-factorial' disease, that is, NAFLD. When implementing the traditional MedDiet in non-Mediterranean countries emphasis should also be placed on customary cooking methods and lifestyle regimen to truly understand the wholesome dietary pattern and its most beneficial components, and to identify whether it is feasible to advise across multi-ethnic populations.

1.3 The impact of diet on inflammation in NAFLD

Whilst the impact of dietary intervention and physical activity has been extensively researched and reported for hepatic and cardiometabolic outcomes, is not well established what the role of these interventions has on inflammatory outcomes. The extent to which dietary patterns improve or worsen an inflammatory state may be an important link to identifying the mechanism by which they contribute to the development and progression of NAFLD.[296] Elucidating dietary sources responsible for pro- and anti-inflammatory cytokine or adipokine release will inform a more appropriate macronutrient composition and/or approaches to dietary guidelines, to obtain potential benefits in terms of reduced inflammation and incidence of NAFLD. Furthermore, finding additional markers that allow for the evaluation of the degree of liver steatosis or systemic disease severity is important since the diagnosis of NAFLD is currently invasive or expensive. To identify whether the MedDiet, or other healthy dietary patterns, have the potential to lower inflammatory cytokines and adipokines in patients diagnosed with NAFLD the doctoral candidate conducted the following systematic review.

1.3.1 Effect of Dietary Intervention on Inflammatory Markers in patients with NAFLD: A Systematic Review

Appendix 1 contains a published systematic literature review titled *"The effect of dietary intervention with or without co-interventions, on inflammatory markers in patients with non-alcoholic fatty liver disease",*[256] published in the journal *Nutrition Reviews, Impact Factor 6.500.*

PUBLICATION

Reddy, A. J., George, E. S., Roberts, S. K., & Tierney, A. C. (2019). Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with non-alcoholic fatty liver disease: a systematic literature review. *Nutrition Reviews*, *77*(11), 765-786. https://doi:10.1093/nutrit/nuz029

Statement of Authorship

The Doctoral Candidate (AR) conducted the systematic literature search of electronic databases and independently completed screening of the retrieved articles based on title and abstract. AR and co-author EG independently assessed the full text of potentially eligible articles. AR independently assessed the quality of eligible studies and extracted data from the included articles. AR, EG and AT assisted with interpretation of data and AR synthesised the results and drafted the manuscript. All co-authors critically reviewed and edited the manuscript.

Permission

Oxford University Press, the publisher of this article, states that the author of this article retains the right to include the article in full or in part in a thesis or dissertation, provided that this is not published commercially. Therefore, permission was not requested.

Supplementary Materials

Supplementary materials which have been published in association to this article are provided in the publication **(Appendix 1)**.

1.3.2 Update to the Systematic Literature Review: Effect of Dietary Intervention on Inflammatory Makers in patients with NAFLD

A search for any relevant articles published between January 15, 2018, and November 30, 2020, was performed by the doctoral candidate. January 15, 2018, is indicative of when the last search was run for the aforementioned, published review by Reddy et al., (2019). Thus, the date limit was applied to capture any articles published since the date of the last search. Relevant databases including MEDLINE Ovid (1946–present), EMBASE Ovid (1947–present), CINAHL (EBSCO), and the Cochrane Library (Wiley Online Library) were searched using the same search strategy and eligibility criteria as was published in the doctoral candidate's systematic literature.[256] A total of 1,733 results were retrieved from the database search and 61 were removed as duplicates. Fifty-six full-text articles were screened, and ten studies were deemed eligible based on the inclusion criteria. All ten studies were randomised controlled trials (RCTs); one was non-blinded,[297] one was double-blinded,[298] five were double-blind placebo controlled,[299-303] two were parallel-group double-blinded,[304] and one was open-label, parallel arm.[305]

Of these ten studies, one study compared a soy-protein meal replacement regimen (MR-G) with a lifestyle-change intervention (LC-G),[297] four compared a hypocaloric diet with a hypocaloric diet plus supplementation (probiotic, sumac powder, flaxseed oil versus sunflower oil and [298, 301, 302] and four studies compared an energy-balanced diet with an energy balanced diet plus supplementation (prebiotic versus probiotic, nigella sativa seed powder, ginger, and flaxseed, hesperidin, or a flaxseed-hesperidin mix).[299, 300, 303, 305] One study placed all participants on a "healthy diet" with particular focus on adherence to a Mediterranean Diet,[306, 307] and moderate physical activity (PA) and randomised participants to receive either a "active nutraceutical" mix (which contained fish oil (70 % docosahexaenoic acid), phosphatidylcholine concentrated in sunflower oil, silymarin, choline bitartrate, curcumin and D-α-tocopherol), or a control (which contained formulation excipients and matched amounts of choline in the form of bitartrate salt).[308] A trial with four intervention arms compared an energy restricted high-protein diet (HPD) supplemented with β-cryptoxanthin (BCX), an energy restricted HPD plus placebo, a standard energy-restricted diet supplemented with BCX and a standard energy-restricted diet plus placebo.[304] Study characteristics, patient population, study design and prescribed interventions are presented in **Table 1.1**. The length of interventions ranged from 12-weeks to 12-months and dietary intervention protocols varied. The nutrient composition, range of prescribed calorie intake, and PA recommendations are detailed in **Table 1.2.** Calorie-restricted diets were either defined as 30% reduction in total calorie intake,[301] or 500-kcal-per-day deficit.[298, 302] The energy-balanced diet and PA guidelines used in four studies were according to Clinical Guidelines for the treatment of Overweight and Obesity.[309] The Mediterranean diet protocol was based on Abenavoli et al. (2018),[306] and PA recommendations were based on a comparative analysis of guidelines for the management of NAFLD.[307]

Table 1.1. Characteristics of studies included for the updated systematic review investigating the effects of dietary intervention(s), with or without co-intervention, on inflammatory markers in adults with non-alcoholic fatty liver disease

Reference	Country	Diagnostic method	Sample, n	Study design/LOE, Qual. Ax	Diet of Interest	2º Diet of Interest	3º Diet of Interest	4º Diet of Interest	Int. length	Inflammatory biomarkers measured
Behrouz et al. (2020)[299]	Iran	US and ALT	Enrolled (n=111), completed and analysed (n=89)	RCT/Level ll, Positive	Energy-balanced diet plus probiotic supplement and prebiotic placebo (n=30)	Energy-balanced diet plus prebiotic supplement and probiotic placebo (n=29)	Energy-balanced diet plus prebiotic and probiotic placebo (n=30)		12 wk	hs-CRP
Cerletti et al. (2020)[308]	Italy	US and elevated levels of either; ALT, AST or GGT	Enrolled (n=126), analysed (n=113)	RCT/Level ll, Positive	Mediterranean diet, physical activity plus "active nutraceutical mix" (n=55)	Mediterranean diet, physical activity plus placebo (n=58)			12 wk	hs-CRP
Darand et al. (2019)[300]	Iran	Fibroscan using CAP score >263 dB/m	Enrolled (n=50), analysed (n=43)	RCT/Level ll, Positive	Energy-balanced diet plus 500mg Nigella sativa seed powder (2g per day)(n=22)	Energy-balanced diet plus (starch as) placebo (n=21)			12 wk	hs-CRP, TNF-α, NF-KB
Deibert et al. (2019)[297]	Germany	US and ALT of >20% the ULN	Total (n=22)	RCT/Level ll, Positive	Meal replacement group (MR-G)(n=11)	Lifestyle change group (LC-G)(n=11)			24 wk	Leptin, adiponectin, resistin, vaspin, fetuin A
Duseja et al. (2019)[301]	Northern India	Liver Bx and AST and ALT (>1.5 times normal limit for >3mo)	Enrolled (n=39), completed and analysed (n=30)	RCT/Level ll, Positive	Hypocaloric diet plus probiotic supplement (n=17)	Hypocaloric diet plus placebo (n=13)			12 mo	Leptin, adiponectin, TNF-α, IL−1β, IL-6, IL-8

Haidari et al. (2020)[304]	Iran	US	Total (n=92)	RCT/Level ll, Positive	Energy-restricted High Protein Diet (HPD) plus β-cryptoxanthin (BCX) supplement	Energy-restricted High Protein Diet (HPD) plus placebo	Energy-restricted standard protein diet plus β-cryptoxanthin (BCX)	Energy-restricted standard protein diet plus placebo	12 wk	hs-CRP, IL-6 CK18-M65, adiponectin
Kazemi et al. (2020)[302]	Iran	Fibroscan (grade >4 kPa) and ALT (>1.5 x upper limit of normal)	Total (n=84)	RCT/Level ll, Positive	Energy-deficit diet plus 2000mg sumac powder per day (n=42)	Energy-deficit diet plus placebo (n=42)			12 wk	hs-CRP
Rafie et al. (2020)[303]	Iran	US and ALT (M >30 U/L, F >19 U/L)	Enrolled (n=50), completed and analysed (n=46),	RCT/Level ll, Positive	Energy-balanced diet plus 3 capsules of 500mg ginger supplement per day (n=23)	Energy-balanced diet plus 3 capsules of 500mg placebo per day (n=23)			12 wk	hs-CRP, TNF-α adiponectin
Rezaei et al. (2020)[298]	Iran	US	Total (n=68)	RCT/Level ll, Positive	Hypo-energetic diet and flaxseed oil (n=34)	Hypo-energetic diet and sunflower oil (n=34)			12 wk	IL-6
Yari et al. (2020)[305]	Iran	Fibroscan (>37% hepatic fat content (CAP ≥ 260, grade ≥ 2))	Enrolled (n=10), analysed (n=92)	RCT/Level ll, Positive	Energy-balanced diet plus flaxseed supplement (n=24)	Energy-balanced diet plus hesperidin supplement (n=22)	Energy-balanced diet plus flaxseed-hesperidin supplement (n=25)	Energy-balanced diet plus placebo (n=21)	12 wk	hs-CRP, TNF-α, NF-KB

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Ax, assessment; BCX, β-cryptoxanthin; Bx, biopsy; hs-CRP, high sensitivity C-reactive protein; HPD, High-Protein Diet; IL-1β, interleukin 1-beta; IL-6, interleukin 6; IL-8, interleukin 8; LC-G, lifestyle-change group; LOE, level of evidence; MR-G, meal replacement group; MR-S, magnetic resonance–spectroscopy; NAFLD, non-alcoholic fatty liver disease; NF-KB, nuclear factor kappa B; RCT, randomized controlled trial; TNF-a, tumor necrosis factor-alpha; US, ultrasound.

Table 1.2. Dietary intervention protocol data extracted from each study included in the update to systematic review

Ref	Diet label	Nutrient Composition Targets	Caloric intake recommendations	Main food sources	Physical activity recommendations
Behrouz et al. 2020[299]	Energy-balanced	52% to 55% of energy as carbohydrate, ≤30% of energy as fat (15% as monounsaturated fatty acids, 5% as polyunsaturated fatty acids, 10% as saturated fatty acids, and <300 mg/day as dietary cholesterol), 15% to 18% of energy as protein, and 20 to 30 g/day as fibres. Diets designed based on the NHLBI Obesity Int Program	~500 to 1000 kcal/d reduction from usual intake	ns	Participants were also offered to exercise ≥30 min, three times/week.
Cerletti et al. 2020[308]	Mediterranean	During the recruitment visit, all subjects received appropriate recommendations about dietary and physical activity lifestyle, according to each site protocols. Recommendations mainly focused on physical activity and healthy diet, in particular through adhesion to Mediterranean diet**.**	ns	ns	Recommendations mainly focused on physical activity and healthy diet, in particular through adhesion to Mediterranean diet.
Darand et al. 2019[300]	Energy-balanced	The distribution of nutrients in relation to the total energy value was as follows: total fat, ≤30 %; total energy value, SFA, 10 %; MUFA, 15 %; PUFA, 5 %; protein, 15–18 %; carbohydrates, 52–55 %; dietary cholesterol,	~500 to 1000 kcal/d reduction from usual intake	ns	Participants were advised to exercise for at least 30 min, three times per week.
Deibert et al. 2019[297]	Lifestyle change group (LG-G); Moderate fat, nutrient-balanced diet	Diet in accordance with the guidelines set by the German Society of Nutrition and the German Society of Sports Medicine and Prevention. The prescribed diet consisted of 1200 to 1500 kcal per day for women and 1500 to 1800 kcal per day for men. It contained approximately 50%-55% of the calories from carbohydrates, 25%-30% from fat, and 15%-20% from protein.	The prescribed diet consisted of 1200 to 1500 kcal per day for women and 1500 to 1800 kcal per day	ns	Physical exercise was performed as a group session once a week during the first 6-weeks and twice per week thereafter. Each participant was instructed to walk mainly at a specific heart rate reflecting 60%-75%

					of the individual estimated VO2max.
	Meal replacement group (MR-G); Hypocaloric diet	First 6-weeks, subjects instructed to replace two daily meals with a commercially available soy yogurt-honey preparation (Almased®). In the following 18 wk, one daily meal was replaced by the preparation. In addition, dietary intake of fat during second phase was not to exceed 60g per day.	The first 6-wk diet contained 1000 kcal per day for women and 1200 kcal for men, while in the following weeks the dietary program was aimed at a maximum of 1500 kcal for women and 1700 kcal for men.	ns	ns
Duseja, et al. 2019[301]	Hypocaloric	Pts overweight/obese, advised 5%–10% of weight reduction (not more than 1.6 kg/week) via hypocaloric diet (30% reduction in calorie intake) by reducing the intake of both carbohydrates and fats.	ns	ns	All patients were advised regular exercise like brisk walking, jogging, running, swimming, cycling, etc for at least 30–45 min/day, for at least 5 days per week.
Haidari et al. 2020[304]	High protein OR normal protein	The percentage of daily energy intake from carbohydrate, fat, and protein in HPDs prescribed to the subjects in the HPD and HPD-BCX groups was approximately 45%, 30%, and 25% respectively, whereas the corresponding values in normal-protein diets prescribed to those in the control and BCX groups were almost 55%, 30%, and 15%, respectively. In all study groups, animal and plant sources of protein each contributed to approximately 50% of total dietary protein intake.	500 k/cal per day (2092 kJ/day) deficit, individually tailored for each participant	ns	ns
Kazemi et al. 2020[302]	Energy-balanced	55–65% carbohydrate, 20–30% from fat, and 10–15% from protein. Diets designed based on the NHLBI Obesity Int Program .	~500 to 1000 kcal/d reduction from usual intake	ns	All pts were advised moderate-intensity PA 30 min per day 5 days p/w

Rafie et al. 2020[303]	Energy-balanced	Both groups were advised to follow an energy balanced diet, according to guidelines published by the North American Association. *Macronutrient breakdown not provided in article.*	~500 to 1000 kcal/d reduction from usual intake	ns	All patients were asked to exercise at least three times a week for 30 mins a day
Rezaei et al. 2020[298]	Hypo-energetic	−2092 kJ/d diet and 20 g/d of the corresponding oil. All participants received a 2092 kJ/d energy-deficit diet, composed of 50–55 %energy from carbohydrates, 10–15 %from protein and 30–35 %from fat.	−2092 kJ/d	To minimise confounding effect of dietary n-3 fatty acids, participants were asked to abstain from fish, nuts, soya products and soya oil during the intervention. Also, they were recommended to consume low-fat meat and dairy products and use baking or grilling instead of frying as the method of cooking.	Patients advised to complete 30-40 min moderate physical activity per day.
Yari et al. 2020[305]	Energy-balanced	Diet was included the following distribution of nutrients in relation to the total daily caloric value: less than 30% of total energy as fat (10% as saturated fatty acids (SFAs), 15% as monounsaturated fatty acids (MUFAs), and 5% as polyunsaturated fatty acids (PUFAs)), 15–18% as protein, 52–55% as carbohydrate, less than 300 mg dietary cholesterol, and 20–30 g fibre, but no further weight loss regiment was provided.	~500 to 1000 kcal/d reduction from usual intake	ns	All pts received lifestyle intervention; dietary modification and 150 min per week medium intensity aerobic exercise.

Abbreviations: kJ, kilojoule; LC-G, lifestyle-change group; MR-G, meal-replacement group; MUFA, monounsaturated fatty acid; NHLBI, National Heart, Lung, and Blood Institute; ns, not specified; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Cytokines most commonly analysed were hs-CRP, TNF-α, IL-6 and NF-κB. hs-CRP was investigated in seven studies.[299, 300, 302-305, 308] hs-CRP significantly decreased (p <0.05) from pre- to post-intervention following an energy-balanced diet supplemented with a prebiotic, probiotic and no supplement (diet alone),[299] 2-grams per day of nigella sativa seed powder,[300] ginger,[303] and hesperidin or flaxseed-plus-hesperidin.[305] A hypocaloric diet supplemented with 2000 mg per day of sumac or no supplement (diet alone) both decreased hs-CRP significantly (p <0.05) from pre-to-post intervention.[302] Cerletti and colleagues (2020) did not observe any change in hs-CRP following the Mediterranean diet recommendations with or without the "active nutraceutical" mix.[308] Haidari et al. (2020) found that an energy restricted HPD plus BCX, an energy restricted HPD plus placebo, a standard energy-restricted diet plus BCX and a standard energy-restricted diet plus placebo (control group) all significantly reduced hs-CRP at 12-weeks.[304] The energy restricted HPD plus BCX group achieved significantly (p <0.05) greater reductions in hs-CRP than the control group.[304]

TNF-α was analysed in four studies.[300, 301, 303, 305] Levels of TNF-α significantly decreased (p <0.05) following a hypocaloric diet plus probiotic supplement,[301] an energy-balanced diet with or without nigella sativa seed powder,[300] and an energy-balanced diet alone or with hesperidin or flaxseed-plus-hesperidin.[305] IL-6 was investigated in three studies.[298, 301, 304] Circulating IL-6 significantly decreased (p <0.05) following a hypocaloric diet plus probiotic supplement.[301]A hypocaloric diet with flaxseed oil or sunflower oil supplement did not significantly change IL-6.[298] IL-6 decreased significantly (p <0.05) after each of the energy restricted HPD plus BCX, an energy restricted HPD plus placebo, a standard energy-restricted diet plus BCX and a standard energy-restricted diet plus placebo (control group).[304] Similar to hs-CRP, the energy restricted HPD plus BCX group achieved significantly (p <0.05) greater reductions in hs-CRP than the control group.[304]

Two studies analysed NF-κB.[300, 305] An energy-balanced diet supplemented with nigella sativa significantly decreased (p <0.05) NF-κB.[300] NF-κB significantly (p < 0.05) decreased following an energy-balanced diet with flaxseed, hesperidin and flaxseed-plus-hesperidin supplements but not after diet alone.[305]

Adiponectin was investigated in four studies.[297, 301, 303, 304] Adiponectin was the most commonly analysed adipokine, and leptin, resistin, vaspin and fetuin A were also reported in one study.[297] This study found that the soy-protein MR-G and LC-G significantly lowered leptin (p <0.05), while adiponectin significantly increased (p <0.05) following the MR-G group only. Resistin, vaspin and fetuin A did not change from pre- to post-intervention in either group. In another study, adiponectin significantly improved (p <0.05) following an energy restricted HPD plus BCX, an energy restricted HPD plus placebo, a standard energy-restricted diet plus BCX and a standard energy-restricted diet plus placebo (control group).[304]

Overall, the ten additional studies included in the updated systematic literature review substantiate findings from Reddy et al. (2019),[256] in that the diets which elicit the most favourable changes in

inflammatory markers were isocaloric or hypocaloric and included recommendations for moderate PA. In these studies, dietary intervention alongside nutraceutical supplementation, particularly BCX, hesperidin or hesperidin-flaxseed and nigella sativa seed powder produced additional benefits to mediating inflammatory markers hs-CRP, TNF-α, NF-κB, IL-6 and adiponectin compared to diet alone.

1.3.3 Mediterranean Diet and Inflammatory Status in NAFLD

The preceding literature review identified that certain diets and co-interventions may be able to mediate the synthesis and release of inflammatory cytokines, which may be vial in mitigating metabolic dysfunction and the development of NAFLD. Though there are limited intervention studies investigating the efficacy of a MedDiet on inflammatory makers, cardio-protective effects of the MedDiet have been reported in the literature, attributed to the anti-inflammatory and anti-oxidant properties of the diet.[310, 311]

Findings from the large-scale PREDIMED study conducted in Spain were influential in supporting the benefits of the MedDiet compared to the LFD on inflammatory markers in a primary prevention setting.[312] C-reactive protein was significantly reduced following a 12-week MedDiet + EVOO intervention, and IL-6 was significantly reduced following both the MedDiet + EVOO and MedDiet + Nut interventions compared to increases in IL-6 observed in the LFD group.[312] Greater adherence to the MedDiet reduced endothelial and monocytary adhesion molecules and chemokines, compared with increases in the LFD group. Of note, participants in MedDiet groups of this study followed an *ad libitum* approach to diet and anti-inflammatory changes were observed in the absence of significant weight loss.[312] In Greece, a 6-month nonrandomised, single-arm and open-label intervention study recruited 44 NAFLD patients with nonsignificant fibrosis and provided nutritional counselling intended to increase adherence to the MedDiet. Following intervention, alongside increased MedDiet adherence, levels of CRP were significantly increased by 73.9% ($p < 0.0001$). No significant effects were observed for leptin, IL-6, and TNF-α ($p > 0.05$), whereas visfatin – a hormone whose plasma concentrations are associated with obesity, T2DM and the MetS – significantly improved at 6-months ($p < 0.05$).[313] These changes were accompanied by significant improvements in liver imaging and fibrosis score, indicating that the MedDiet pattern may be beneficial for NAFLD as a systemic disease, and not just a hepatic disease.[313]

Individual components of the MedDiet are thought to work synergistically to provide anti-inflammatory effects, which may slow or reverse oxidative stress. A common feature of dietary patterns thought to reduce inflammation is an increased intake of functional foods which contain essential bioactive compounds known to provide health benefits. Olive oil, particularly in the form of EVOO, retains lipophilic components of the olive fruit, phenolic compounds and antioxidants, and smaller quantities of alpha-tocopherol and phytosterols.[314] High intake of virgin olive oil and nuts have been associated with lower concentrations of IL-6, CRP and inflammatory markers

related to endothelial function; VCAM-1 and ICAM-1.[314] Olive oil and nuts have favourable fatty-acid profiles, and besides being rich in unsaturated fatty acids, nuts are high in fibre, phenolic compounds and antioxidants [315, 316] and intakes are inversely associated with lower concentrations of inflammatory markers in multiethnic populations.[317, 318]

Some dietary components of the MedDiet overlap with similar healthy dietary patterns, however there are many aspects of the diet which are unique to the traditional 'Cretan' MedDiet Vegetables and fruits are important sources of bioactive compounds including phenols, flavonoids and phytosterols, as well as fibre, antioxidants, vitamins and minerals.[319] Diets with high content of antioxidants have been associated with reduced CRP concentrations, as compared with diets with low antioxidant content.[320] High consumption of vegetables and fruit has been associated with lower concentrations of CRP [321] and IL-6,[314] and dietary vitamin C intake has been inversely associated with CRP.[322] Long chain n-3 PUFA content of fish has been associated with lower concentrations of IL-6, CRP and markers of endothelial dysfunction.[323, 324] Fermented dairy products recommended in the MedDiet such as yoghurt and feta cheese may confer probiotic benefits and therefore improve gastrointestinal and immune health.[319]Although there is some evidence of an inverse relationship between a higher consumption of dairy foods and lower levels of CRP, the mechanisms explaining these associations are not well understood.[314] Similarly, there is limited and somewhat contradictory evidence for the effects of wholegrain intake on inflammation. A few small-scale intervention studies have reported lower concentrations of CRP and IL-6,[325] though additional studies assessing dose-response relationships are required.

The proposed mechanisms by which diet influences inflammation, oxidative stress and insulin sensitivity in NAFLD are summarised in the **Figure 1.5** (below). Key cytokines CRP, TNF-α, IL-6, adiponectin and resistin are produced via adipose tissue proliferation initiated by 'unhealthy' or western dietary patterns. Conversely, these cytokines may be modulated by a healthy diet to induce anti-inflammatory effects, improved endothelial function and insulin sensitivity and decrease uptake of triglyceride into the liver causing NAFLD and/or progression to NASH.[326]

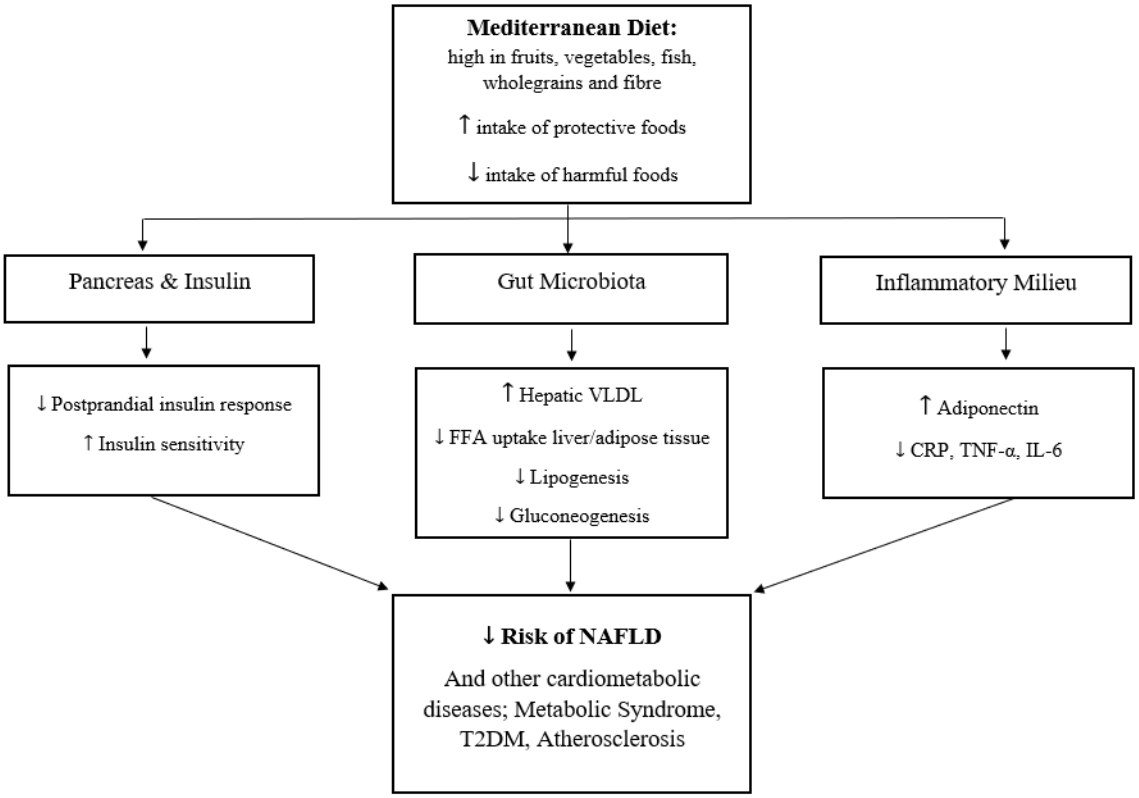


Figure 1.5. Proposed anti-inflammatory mechanisms of the Mediterranean diet and resultant benefit for NAFLD and other cardiometabolic diseases. *Adapted from Mirmiran et al. (2017) and Esposito et al. (2017)*[326, 327]

1.3.1 Evidence for Gene-Diet Interaction in NAFLD

The study of gene-nutrient interaction in humans is a developing area of science, termed nutrigenetics and nutrigenomics. Nutrigenetics refers to the study of how an individual's genetic background, considering underlying genetic polymorphisms, impacts their response to diet.[328] Put differently, nutrigenetics represents the science of identifying and understanding the impact of gene variants' differential responses to nutrients, and characterise these responses in relation to disease states.[328] Nutrigenomics, on the other hand, aims to elucidate the effects of micro- and macro-nutrients or bioactive food components on the genome (**Figure 1.6**).[329] Nutrigenomics aims to understand the influence of nutrients as stimuli to transcription activity, gene expression and heterogenous response of gene variants or control of metabolic pathways and homeostatic processes.[328, 329]

Nutrients are able to impact molecular mechanisms occurring after ingestion, modulating physiological functions in the body. Genetic polymorphisms may ultimately influence the manner in which enzymatic activities and circulating concentrations of metabolites leading to pathogenesis or progression of disease.[330] In NAFLD, the hepatic lipase gene (HL) which regulates triglyceride levels in response to an up-regulation of insulin, has been associated with gene-nutrient interactions of HL polymorphism -514C>T and total dietary fat and saturated fat.[331] Dietary fibre intake was also associated with HL activity in an epidemiologic study of 42 SNPs in 26 candidate genes.[332]

The apolipoprotein A5 (APOA5) gene, an important regulator of plasma triglyceride level, has been linked with dietary fat in determining plasma fasting triglycerides after ingestion.[331] Studies concluded that the ratio of n-6 to n-3 PUFAs (n-6 PUFA-rich diet) were linked to higher levels of triglycerides in carriers of the -1131C polymorphism in APOA5 gene.[333] A similar finding was observed for PNPLA3 genotype rs738409, wherein the ratio between n-6 to n-3 PUFAs was linked to increased hepatic steatosis.[334] These are the main gene-diet interactions which may infer susceptibility to NAFLD. Studies investigating the effect of diet or nutrient interaction with genes influencing inflammation in NAFLD are scarce. There is a need to understand the biological impact of gene-diet interactions for inflammatory processes which may underlie the pathogenesis and progression of NAFLD, a diet-related polygenic disorder.

Identifying genetic factors that predispose an individual to higher rates of inflammation or higher production of cytokines in response to dietary intake, may allow preventative strategies to be targeted at individuals at higher risk.[183] Presently, a limitation of lifestyle and dietary interventions in the management of many chronic diet-related diseases is the individual variation in response to a given therapy. Some individuals may achieve the desired outcome by following dietary recommendations, whereas some individuals are unable to achieve an adequate outcome based on the same diet prescription.[328] Moreover, compliance to dietary prescription presents another barrier to determining the true effect of diet and compliance is generally poorly reported in most dietary intervention trials. The subsequent design and delivery of dietary intervention trials should consider strategies to improve and more accurately measure dietary compliance. Variations in the degree of response to diet is largely accredited to genetic factors, especially in a NAFLD population, which emphasises the importance of elucidating and characterising the genes that play a crucial role in promoting inflammation in individuals with and without NAFLD.[328] Insights into the interactions of genes with nutrients will optimise the ability to recommend novel, targeted diet interventions and personalised nutrition recommendations for the management and prevention of disease.

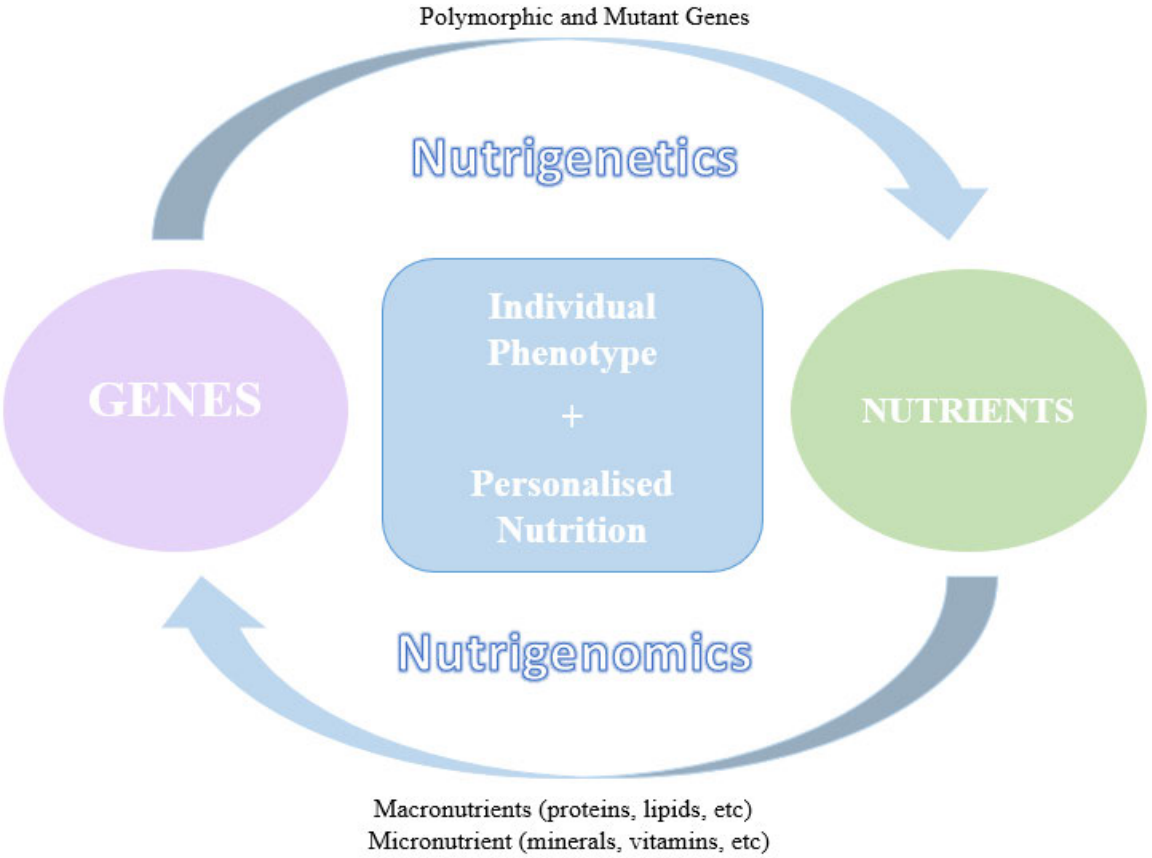


Figure 1.6. Nutrigenetics and Nutrigenomics stem from the interaction between genes and nutrients. *Adapted from Farhud, Yeganeh and Yeganeh (2010).*[329] Nutrigenetics encapsulates the study of how the genetic background of an individual impacts their response to diet and environmental factors, while nutrigenomics is the study of the effects of several nutrients, including macronutrients and micronutrients, on the genome.

1.4 Dietary Inflammatory Index

In measuring adherence to dietary patterns, tools or scores that assess individual food components which provide health benefits have traditionally been used. For example, adherence to the MedDiet can be assessed using a number of tools that were primarily designed to assist nutrition epidemiology studies to identify associations between diet adherence and development (or risk) of chronic disease.[335] However, these tools do not examine the degree to which diet may induce or supress inflammatory pathways, or otherwise put, assess the dietary inflammatory potential.[336] Assessment of dietary inflammatory potential recognises complex interactions between components of diet and food items that may impact inflammation differently. The Dietary Inflammatory Index (DII®) characterises certain foods by their potential to influence underlying biological mechanisms which have a known effect on markers of inflammation. The score is based on a calculation of each food item's inflammatory potential, and together the individual components are used to calculate the overall pro- or anti-inflammatory potential of the diet. The DII may provide

additional insight into the theoretical measure of inflammatory potential of a MedDiet, rather than a simple measure of adherence to the general dietary pattern.

1.4.1 Development and Validation of the DII

The DII was designed as a scoring algorithm to categorise an individuals' diet based on theoretical inflammatory potential that was derived from the literature. The DII provides a novel tool to assess whether the dietary pattern is maximally anti-inflammatory, maximally pro-inflammatory or anyplace in between. Six inflammatory markers: IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP were chosen to be the focus of a literature search, based on established and robust existing scientific evidence for their important role in inflammation.[337] A total of 1943 published studies that reported associations between nutritional factors and inflammatory cytokine markers qualified for analysis.[338] From these studies, a total of 45 food parameters were identified which constitute the DII: energy, carbohydrate, protein, alcohol, fibre, total fat, cholesterol, SFA, TFA, MUFA, PUFA, omega-3, omega-6, niacin, thiamin, riboflavin, selenium, vitamin B6, vitamin B12, iron, magnesium, zinc, vitamin A, vitamin C, vitamin D, vitamin E, folic acid, beta carotene, caffeine, eugenol, anthocyanidins, flavan-3-ol, flavonols, flavonones, flavones, isoflavones, garlic, ginger, onion, pepper, saffron, turmeric, rosemary, thyme/oregano and black/green tea.[338] **Appendix 2** summarises the 45 food parameters included in the DII, DII, inflammatory effect scores, and intake values from the global composite data set - as reported in the Dietary Inflammatory Index Development Study, Columbia, SC, USA, 2011–2012.[338]

A consistent scoring algorithm was applied to all articles. One of three values were assigned to each food parameter based on its impact on inflammation: '+1' was assigned for a pro-inflammatory change in marker (significant increase in IL-1β, IL-6, TNF-α or CRP, or significant decrease in IL-4 or IL-10); '-1' for an anti-inflammatory change in marker (significant decrease in IL-1β, IL-6, TNF-α or CRP, or significant increase in IL-4 or IL-10); and '0' where there was no change in inflammatory marker.[338] In the case of a single study reporting the differential effects of one food parameter (i.e., a food parameter increasing one (pro-)inflammatory marker while decreasing another one), separate scores were assigned for each effect reported in the same article. Dietary intake data derived from nutritional databases of eleven countries around the world were standardised, then transformed into percentiles and multiplied by the inflammatory scores for each food parameter to obtain the 'food parameter-specific DII score'. The sum of all the individual 'food parameter-specific DII scores' creates the 'overall DII score'. The DII score can range from a minimum of -8.9 to a maximum of +8, with a negative DII score indicating lower (or anti-inflammatory) inflammatory potential of diet and a positive DI score indicating higher (pro-inflammatory) inflammatory potential of diet. The DII score was validated using data from the Seasonal Variation of Blood Cholesterol (SEASONS) study, which confirmed that higher DII scores, derived from 24-hr recall and 7-day food records, were associated with levels of CRP

>3mg/L in 519 healthy participants (OR = 1.08; 95% CI 1.01, 1.16, P = 0.035 for data derived from 24-hr recall; and OR = 1.10; 95% CI 1.02, 1.19, P = 0.015 for data derived from 7-day dietary recall).[339] Subsequently, the DII has been validated in a large observational study of post-menopausal women which reported that FFQ-derived DII significantly predicted higher levels of circulating IL-6 and a cross-sectional study of people with asthma, findings that those with asthma had a higher DII score than healthy individuals.[340, 341] The latter study found that people with asthma had higher levels of cytokine IL-6 and FEV^1 which were associated with DII score.[341] Another study in healthy individuals reported no association between DII and levels of CRP, though a significant association was observed for younger participants only (results of an age-adjusted analysis).[342]

1.4.2 Application of DII in NAFLD

Presently, the DII has been associated with fatty liver and liver-related markers in two cross-sectional studies.[343, 344] A National Health and Nutrition Examination Survey (NHANES) study of 20,643 individuals in the US demonstrated an association between higher DII and fatty liver index (FLI) score, and liver enzymes ALT and AST.[344] Interestingly, these associations were only observed among individuals with lower BMI, but not among those classified as obese after adjustment for as a covariate. An analysis of a subset of the PREDIMED trial cohort showed that a higher DII score was positively associated with non-invasive liver markers (FLI, ALT, AST and GGT) and negatively associated with MedDiet adherence.[343] This analysis also found that higher DII scores were found in individuals with obesity and lower adherence to the MedDiet.[343] There is currently no published literature investigating the association between DII score and hepatic steatosis or liver histology in patients diagnosed with NAFLD, nor assessing the impact of dietary intervention on DII score in NAFLD.

1.5 Summary of Chapter 1

Rates of NAFLD are forecasted to rise in parallel with obesity and T2DM and in the absence of proven safe and effective pharmacotherapy, diet and lifestyle intervention remain the first-line therapeutic option for management of the disease. No evidence-based dietary recommendations exist for NAFLD, beyond the scope of energy-restriction and weight loss which are unattainable and/or unsustainable in this patient group. The MedDiet is evidenced to provide beneficial metabolic and anti-inflammatory effects, without the intention of weight loss, in patients with the metabolic syndrome and diabetes, as well as cardiovascular diseases. Inflammation is well-recognised in the pathogenesis of NAFLD, although the effects of a MedDiet on inflammatory markers in this patient group is unclear. Moreover, clinical trials assessing the translation of an *ad libitum* MedDiet in a multicultural, free-living Australian population are lacking. Understanding the effects of this dietary pattern in patients diagnosed with NAFLD may influence the management of the disease and could be used more effectively slow or stop progression of the disease due to underlying inflammation.

The Dietary Inflammatory Index (DII) is a novel dietary assessment tool which provides a theoretical measure of inflammation derived by food components and nutrients. It is increasingly used as a surrogate measure of inflammation, calculated from an individual's dietary intake. The DII score was developed based on the food item or nutrients' association with a selection of the most researched inflammatory cytokines, and the score has increasingly been associated with prevalence or incidence of diet-related diseases in epidemiological studies. Considering the anti-inflammatory nature of the MedDiet, it is anticipated that the MedDiet will have a lower (more anti-inflammatory) DII score than the control diet (the low-fat diet) and that participants following this diet will reduce their DII score to a greater extent than participants in the control group. There are currently no RCTs investigating DII as a surrogate measure of dietary intervention in patients with NAFLD. Genetics are a large predictor of the development of NAFLD, and specific genes are well-known to confer risk. Gene polymorphisms that influence inflammation in this population are not as well understood, and the impact of gene-nutrient interactions which may produce harmful increases in pro-inflammatory cytokines is an area which requires further exploration. The influence of an individual's genetic makeup on inflammation, known to progress disease state, in nutritionally related disease such as NAFLD must be considered in the management of disease.

1.6 Thesis Preface

The **Med**iterranean **Di**etary **I**ntervention in patients with **N**on-**A**lcoholic Fatty Liver Disease (MEDINA) study was a randomised controlled trial (RCT) that aimed to investigate the effects of a Mediterranean Diet (MedDiet) compared to a low-fat diet (LFD) in the management and/or reversal of NAFLD. The a 12-week parallel RCT which recruited participants from outpatient liver clinics at three major metropolitan hospitals in Melbourne, Australia; Alfred Health, Eastern Health and The Royal Melbourne. Patients were eligible to participate in the study if they spoke fluent English, were over the age of 18 years, had biopsy or ultrasound proven NAFLD and were deemed insulin resistant using the homeostatic model of assessment for insulin resistance (HOMA-IR) score ≥2. Patients who were recruited to the study underwent randomisation to either a MedDiet or LFD, where they received dietary counselling, food hampers or vouchers and other intervention-specific dietary resources. This doctoral research was embedded into the MEDINA study and the specific aims and objectives of this PhD are stated below.

1.7 Thesis Aims and Research Questions

Primary research aim

The aim of this PhD research programme was to determine whether a 12-week *ad libitum* Mediterranean Diet can improve markers of inflammation in Australian patients diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) compared to a low-fat diet.

Primary research question

Does a 12-week intervention with an ad libitum MedDiet in Australian patients diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) improve markers of inflammation compared to a low-fat diet? (Chapter 4).

Secondary research questions

1. What is the habitual diet quality and baseline inflammatory and metabolic risk marker profile of Australian patients with NAFLD? (Chapter 3).
2. Does a 12-week intervention with an ad libitum MedDiet, delivered by a Dietitian, in Australian patients diagnosed with Non-Alcoholic Fatty Liver Disease (NAFLD) improve markers of inflammation, body composition, liver outcomes and blood biomarkers, compared to a low-fat diet? (Chapter 4).
3. Does a multi-ethnic cohort of Australians with NAFLD adhere well to a 12-week MedDiet intervention? (Chapter 4).

4. Does adherence to the MedDiet and overall improvement in diet quality elicit greater improvements in inflammatory markers, insulin resistance and liver outcomes in individuals diagnosed with NAFLD and T2DM, compared to those with NAFLD and without T2DM? (Chapter 4)

5. Was there an association between increasing MedDiet adherence score and reduction in inflammatory potential of the diet (as measured by the Dietary Inflammatory Index) following dietary intervention? (Chapter 5).

6. Is dietary-induced reduction in DII score associated with improvement in inflammatory markers in patients with NAFLD? (Chapter 5)

7. What is the prevalence of inflammatory gene polymorphisms CRP +1846, IL-6 -174, TNF-α -308 and adiponectin +276 in this cohort of patients with NAFLD, and are these polymorphisms associated with serum inflammatory marker concentration? (Chapter 6)

8. Does the presence of the gene polymorphism effect average inflammatory marker concentration in response to dietary change? (Chapter 6)

1.8 Thesis Structure

Chapter 1 of this doctoral thesis contains the background, rationale and proposed aim(s) for this research. This introductory chapter begins with an overview of NAFLD and the role of inflammation in disease pathogenesis and progression. This chapter outlines the current and predicted disease burden of NAFLD, current management strategies and reported dietary habits of those diagnosed with NAFLD. A review of various dietary interventions tested in individuals diagnosed with NAFLD leads into the rationale for testing a Mediterranean Diet on outcomes of inflammation in this population. A literature review performed and published by the doctoral candidate is embedded into this section, titled the '*Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with non-alcoholic fatty liver disease: a systematic literature review'*.[256] This systematic review highlights the potential for anti-inflammatory diets in the improvement of underlying chronic inflammation present in NAFLD and in the absence of any known liver-sensitive markers, cytokines and adipokines may be considered potential surrogate markers of liver disease. The core results of this review strengthen the rationale for the doctoral research.

Chapter 2 provides details of the methodology of the overarching Mediterranean Dietary intervention in patients with NAFLD (MEDINA) RCT, including a detailed description of the study design of the trial, recruitment and data collection timepoints, methods and protocol. This chapter is based on the published protocol for the overarching randomised controlled trial by Papamiltiadous et al.[345]

Chapter 3 of this thesis presents baseline data from the MEDINA study. The anthropometric, biochemical, inflammatory and liver outcomes of participants who were recruited to this study were analysed and compared with current literature in other NAFLD populations. Dietary intake at baseline was also assessed. Due to the similar underlying metabolic dysfunction and risk factors between the Metabolic Syndrome (MetS) and NAFLD, participants were grouped on the basis of having the MetS and differences observed. Similarly, sex differences are known to be apparent in NAFLD therefore participants were split by sex and inflammatory markers, biochemistry, liver outcomes and anthropometric and body composition measures were compared at baseline.

Chapter 4 presents the main body of work included in this thesis and addresses the primary aim of this doctoral research programme. The chapter first reports on the effects of a MedDiet versus low-fat diet on inflammation and secondary outcomes in NAFLD, as well as reporting on the change in diet quality over the intervention period and assessing compliance to a MedDiet in the pooled NAFLD cohort (irrespective of original diet group assignment). A subgroup analysis of individuals diagnosed with both NAFLD and type 2 diabetes mellitus was undertaken due to the identification of an uneven number of participants with and without diabetes randomised to each diet group. The rationale for undertaking this analysis is discussed further in the chapter.

Chapter 5 explores the effect of diet change on the Dietary Inflammatory Index (DII score), a tool that was designed by researchers in the US to provide a surrogate, theoretical measure of dietary inflammatory potential. Chapter 6 explores the impact that four genetic polymorphisms may have on inflammation, as it is well known that both genetic and environmental (diet and lifestyle) factors are major determinants in the development and progression of NAFLD. Finally, the concluding chapter discusses the main findings of this doctoral research, the implications of these findings and future recommendations. **Figure 1.7** summarises each chapter and the overall structure of this thesis.

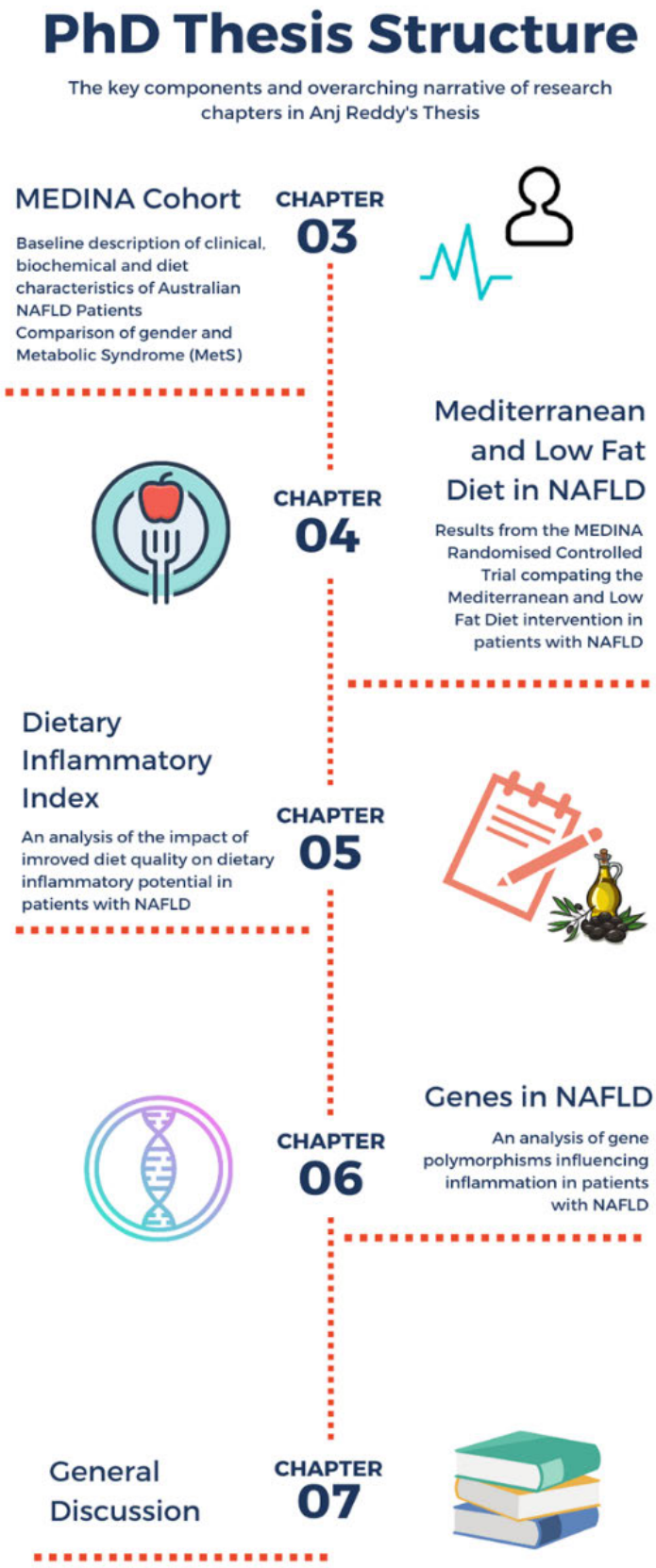


Figure 1.7. A summary of the structure of this doctoral thesis and an outline of the key components of each research chapter.

2 Methods

The "MEDINA" Study: Mediterranean Diet Intervention in NAFLD Patients

2.1 Chapter Overview

This chapter will describe the methodology employed throughout the MEDINA (Mediterranean Diet Intervention in NAFLD Patients) study. Details of the study design, overarching study aims, eligibility, screening and recruitment, dietary interventions, data collection, outcome measures, and statistical analysis will be detailed and discussed. The MEDINA study methods have also been published as a manuscript entitled *"A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non-Alcoholic Fatty Liver Disease (MEDINA): study protocol"*[345] in the journal *BMC Gastroenterology, Impact factor 2.212.*

2.2 MEDINA Study Design

The MEDINA study is a 12-week, multi-centre, parallel, randomised controlled trial in patients with ultrasound or biopsy proven NAFLD and NASH [345]. Participants were recruited from three major metropolitan hospitals across Melbourne, Australia; Alfred Health, Eastern Health and The Royal Melbourne Hospital. Patients attending outpatient Liver Clinics were screened and recruited based on eligibility. Once deemed eligible, the participant was assigned to an experimental group using a computer-generated randomisation stratified to the participant sex and diabetes status completed independently by a senior researcher. There were two diet groups; the dietary intervention group received a Mediterranean Diet (MedDiet) and the control group received a Low-Fat Diet (LFD), also known as the standard care diet for patients with NAFLD. Participants were required to attend three face-to face dietary consultations, lifestyle, anthropometry and biochemistry assessment appointments at baseline (0 weeks), mid-intervention (6 weeks) and end-intervention (12 weeks).[345] Participants from both dietary intervention groups also received phone call follow-up reviews from their respective dietitian during weeks 2, 4 and 9. Although the dietary intervention ran for 3-months, all participants were required to attend a face-to-face follow up appointment at 6- and 12-months post-intervention in order to determine maintenance and sustainability of dietary interventions. Although the overarching MEDINA trial involved the two follow-up timepoints to assess sustainability, only data from baseline to end-intervention (0-weeks, 6-weeks and 12-weeks) are presented in this doctoral thesis.

2.3 MEDINA Overall Study Aims

The primary aims of the broader MEDINA study were to determine the effects of a MedDiet versus LFD on markers of insulin resistance (IR), hepatic steatosis and associated risk factors of the metabolic syndrome (MetS), in patients diagnosed with NAFLD and/or NASH. A secondary aim was to explore the relationship between the molecular mechanisms of the disease and the dietary

interventions. It was hypothesised that the MedDiet would improve insulin resistance, reduce the severity of fatty liver, and that the improvements would be sustained at 12 months post-intervention with maintenance of diet.

Thesis Aims and Hypothesis

The primary aim of this doctorate was to investigate the effects of an *ad libitum* MedDiet versus LFD on circulating serum inflammatory markers in patients diagnosed with NAFLD and/or NASH.

It was hypothesised that the MedDiet would be superior to the LFD in improving inflammatory markers in patients diagnosed with NAFLD.

2.4 Participant Eligibility and Screening

Patients attending outpatient Liver Clinics at the Alfred Hospital, Box Hill Hospital and the Royal Melbourne Hospital that were potentially eligible to participate in the study were referred by their managing hepatologist. A trained researcher assessed patient eligibility using a screening questionnaire, made up of a series of questions to ensure that the individual met the inclusion/exclusion criteria. Details of the MEDINA study eligibility criteria are outlined in **Table 2.1**.

Table 2.1. The MEDINA Study Eligibility Criteria

Inclusion Criteria	**Exclusion Criteria**
>18 years old	Refusal or inability to provide informed consent to participate in the study
Male or female (not pregnant)	Non-English speaking
Smokers and non-smokers	Above average alcohol ingestion (>140g per week for men and women)
Diagnosis of NAFLD based on ultrasound and/or biopsy within 12 months of screening	Weight change exceeding 5kg (loss or gain) within 3 months of screening
Body mass index (BMI) between 20-40 kg/m^2	Currently following or anticipated commencement of a diet (such as Light and Easy, Jenny Craig, etc.)
An elevated serum aminotransferase (ALT) level (>20U/L for females and >30U/L or males) within 6 months of screening	HbA1c >8%
Patient must be insulin resistant, as determined by fasting glucose and insulin used to calculate homeostatic model of assessment insulin resistance (HOMA-IR) score. HOMA-IR Score ≥2.	A diagnosis of Type 1 Diabetes Mellitus or IDDM
No current or past history of cardiovascular, cerebrovascular or peripheral vascular disease	Taking immunosuppressants, amiodarone and/or perhexiline
No evidence of any other form of liver disease (further outlined in exclusion criteria)	Any other cause of chronic liver disease or hepatic steatosis; ALT or AST >10 x ULN at screening or within 3 months of screening
	A current or past history of cardiovascular, cerebrovascular or peripheral vascular disease

	Presence of clinically relevant pulmonary, gastro-intestinal, renal, metabolic, haematological, neurological, psychiatric, systemic or any acute infectious disease or signs or acute illness
	Any psychosocial or gastrointestinal malabsorptive (e.g. coeliac disease) contraindications, including bulimia nervosa, anorexia nervosa, substance abuse, clinically significant depression or current psychiatric care
	Change in dose or introduction of fish oil, probiotics, vitamin E, vitamin C or high dose vitamin D (>3000IU) within 3 months of screening

2.5 Recruitment and Randomisation

Patients who met the initial eligibility criteria were flagged by the researcher and managing hepatologist, and the patient was thoroughly informed about the study in lay terms by the researcher. If the patient agreed to participate in the study, they were asked to sign a Participant Information Consent Form (PICF)**(Appendix 3.1)**, after which they underwent a thorough screening questionnaire (**Appendix 3.2**) which was administered by the researcher.

The PICF contained all of the information regarding overall purpose of the study, aim(s), processes, expectations and requirements, as well as relevant study coordinator contacts information, and a choice of optional measures (such as dual energy X-ray absorptiometry (DEXA) scans) for participants to indicate their interest **(Appendix 3.1)**. Information regarding the storage of participants samples was provided in the PICF, and participants were required to consent to whether or not further analyses could be undertaken on the samples, if required **(Appendix 3.1)**. All consenting participants were to receive a copy of their results sent by the primary researcher, however in most cases the researcher provided these results directly to the participant's general practitioner (GP) and/or referring hepatologist.

The thorough screening questionnaire which was administered face-to-face or over the phone by the primary researcher contained a series of questions confirming and further identifying personal and clinical characteristics of patients addressed in the eligibility criteria. All of the criteria in the screening questionnaire were to be met prior to enrolment in the study.

Once the patient was deemed eligible, their de-identified information was sent to a statistician who completed a computer-generated randomisation stratified to sex and diabetes status. The participant was randomly allocated to either the control (LFD) or intervention (MedDiet) arm of the study, the statistician informed the investigator of the study diet allocation and initial appointments were organised. Allocation concealment, blinding and randomisation ensured that participants were equally distributed to each group, without inadvertent allocation or researcher bias, which could result in confounding differences between groups at baseline. Allocation concealment was deemed

successful, as there were no significant differences in primary outcome measures (hs-CRP, IL-6, TNF-α, adiponectin, leptin and resistin) between the LFD and MedDiet groups at baseline (Chapter 4; Table 4.1, page 178).

Considering that both study groups involved the active delivery and uptake of dietary interventions, blinding of the study condition was not possible for both researchers and participants. Outcome assessors were blinded for specific measures (such as MR-S or Fibroscan), and the relevant methodology stated within the appropriate sections of this thesis.

2.6 Timeline of Appointments

Participants were randomised to either a LFD of MedDiet intervention for twelve-weeks, during which time they were required to attend three face-to-face appointments and three phone-call follow up consultations with an Accredited Practicing Dietitian (APD). The face-to-face appointments occurred at baseline (0 weeks), mid-intervention (6 weeks) and end intervention (12 weeks), and the phone-call follow up consultations occurred at weeks 2, 4 and 9. Once the intervention period ended, all participants were also required to attend face-to-face follow up appointments at 6 and 12 months after study commencement to assess maintenance of diet and sustainability of outcomes after the intervention period. A summary of the data collected, and the corresponding timeline of appointments are listed in **Table 2.3**. Each face-to-face appointment ran for approximately 45-60 minutes in duration, and phone calls were between 10-20 minutes each.

The same meal was provided for breakfast to all participants. Regardless of diet group allocation, following their fasting blood test during each face-to-face appointment. Breakfast consisted of 1x tub of Jalna yoghurt (170g) and 1x muesli bar. This breakfast composition was aligned in both groups.

2.7 Dietary Intervention Arms

Participants allocated to the MedDiet and LFD intervention groups both received the same amount of dietary counselling, educational resources and face-to-face/phone-call follow up time with their respective APD's. The MedDiet dietitian differed from the LFD dietitian and each were trained in the recommendations specific to the intervention they were administering. This was done to ensure there was no repetition or cross-over in dietary advice given to each group and to avoid any bias occurring.

As previously mentioned, participants who were following or anticipating the commencement of any type of diet (Jenny Craig, Optifast, etc.) were excluded and participants were also not allowed to participate if they were seeing a dietitian and receiving dietary advice from an APD other than their allocated APD on the trial. Participants who had previously received dietary advice in the clinical setting or otherwise were not excluded, though often the details of prior dietary advice were noted within the patient file.

2.7.1 Mediterranean Diet

The MedDiet dietary intervention recommendations and nutrition counselling techniques that were administered to participants in this study were specifically developed for the MEDINA trial. The key components and diet model of the intervention were based on a traditional MedDiet as described by Keys et al. (1986) in the seven countries study.[346] The approximate macro and micronutrient composition of the MedDiet was based on previous literature from controlled trials outlined as 44% fat (>50% monounsaturated), 36% carbohydrate and 17–20% protein, and up to 5% alcohol. Traditional attributes of the MedDiet were also established using existing literature, with the inclusion of MedDiet principles such as a high consumption of plant-based foods; fruits, vegetables and wholegrains, with an emphasis on the consumption of legumes, nuts and oily fish, moderate consumption of fermented dairy products and white/game meats, and decreased consumption of red meat and sweetened or processed foods. The main source of culinary fat was extra-virgin olive oil (EVOO).

One of the main considerations when administering the MedDiet intervention to this group was that they were multiethnic living in a non-Mediterranean country and were diverse in age. The traditional Cretan diet and meal options were therefore diversified to include culturally appropriate options which still adhered to the traditional MedDiet principles. This diet was administered as *ad libitum* and dietary counselling was intended to focus on what participants were able to consume rather than what they should avoid. Briefly, the prescribed MedDiet was developed based on a combination of principles derived from the Dietary Guidelines for Adults in Greece[347] and MedDiet patterns and key components described in other pivotal studies.[348-351] Nutrient composition from clinical trials where benefits of intervention were seen (improvements in outcome measures) in a range of chronic diseases or where feasibility in an Australian population was shown, were collated and entered into nutrient analysis software. The nutrient profile and food components recognised for health promoting effects were used to formulate a meal plan. A two-week modelled example of the diet is provided in **Appendix 4** and daily macro- and micronutrient profile that was derived from these menus is reported in **Table 2.2** (below). The MedDiet provides approximately 9,400kJ (or 2,245kcal) of energy and is classified as a 'high-fat' diet with 35-45% total energy from fat, 35-40% energy from carbohydrates and 15-20% energy from protein. Monounsaturated fatty acids (MUFAs) make up 50% of the energy from total fat, and the remaining energy contribution from polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). Alcohol intake may contribute up to 5% total energy in the MedDiet, which is classified as a moderate amount and is within the recommendations set by the Australian National Health and Medical Research Council (NHMRC).[352] Portion sizes were not specifically detailed to participants as this diet was not intended to achieve weight-loss. Development of this MedDiet model are published elsewhere.[1]

Table 2.2. Nutrient composition of the Australian Mediterranean Diet – taken from George et al. 2018.[1]

Nutrients	Australian Mediterranean Composition
Energy (MJ)	9.4
Protein (%E)	15.8
CHO (%E)	33.8
Added sugar (%E)	5.2
Total fat (%E)	41.8
SFA (%E)	8.9
MUFA (%E)	22.3
PUFA (%E)	10.6
Alcohol (%E)	2.4
Fibre (g/d)	41.1
Linoleic acid n-6 (g)	18.7
α linolenic acid n-3 (g)	4.9
Total LCN3s (mg)	932

Energy intake equivalent to 2,245kcal. Two-week food diaries based on the Mediterranean diet meal plan were entered into Foodworks 7™ (Xyris software Australia Pty Ltd.) to calculate this nutrient profile. Abbreviations: CHO, carbohydrates; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, LCN3s, long chain omega-3 fatty acids.

Specific counselling techniques were employed by dietitians when delivering the MedDiet intervention to promote positive reinforcement, mindfulness and patient centred goal setting.[353, 354] As previously stated, due to the ethnic diversity in the population the intervention was designed to follow a traditional MedDiet pattern but also be practical and easy-to-follow while allowing for individual preference and cultural circumstances. The aim was to allow participants to make small, sustainable changes to their diet and positively reinforce these changes. This was thought to be more beneficial than a complete overhaul of the diet, as full adherence to a new diet is often unsustainable, overwhelming and sometimes discouraging for trial participants.[355]

In an effort to increase compliance and motivation toward dietary recommendations and to model the staple foods of the diet,[348] a food hamper containing examples of staple foods captured in the MedDiet were provided to participants at all face-to-face consultations during the intervention period. The first two hampers provided at baseline and 6-week appointments were substantial and provided bottles of EVOO, nuts, tinned fish and legumes, and a recipe book. The final hamper provided to participants at the 12-week appointment was slightly smaller and acted as an incentive to promote attendance for the final intervention appointment. A full list of items provided during each appointment is available in **Appendix 4**, and an example of hamper content from the baseline (and 6-week) appointment is shown in **Figure 2.1**. The food items used in the hamper were donated to the MEDINA Study by various food industry providers who were contacted for particular items.

Figure 2.1. The Mediterranean Diet hamper provided to participants in the MedDiet intervention group at baseline and mid-intervention timepoints

2.7.2 Low-Fat Diet

The LFD intervention was based on the current Australian Dietary Guidelines and Heart Foundation recommendations, which comprise of a macronutrient composition of approximately 30% fat, 50% carbohydrate and 20% protein.[356] The resources and education provided was primarily based on the Australian Guide to Healthy Eating with an emphasis on low-fat cooking methods, portion sizes and information around suitable low-fat options. As previously outlined, participants in the LFD group received the same amount and duration of face-to-face and phone-call follow up consultations with an APD. Dietary advice was delivered by the liver clinic dietitians who consulted trial patients as they would in standard practice, providing the same recommendations, counselling techniques and resources given by the hospital nutrition department. In order to compare effectiveness of the two diets, the LFD was also administered *ad libitum* and dietitians were explicitly asked not to focus on weight-loss as a main outcome or patient goal. Although the diet was kept as a standard practice or 'control' low-fat diet, researchers ensured that patient-dietitian consultation timing and frequency matched that of the MedDiet group so that variability would not create bias and the impact of dietary advice given could be directly compared.

Participants were provided with $20 AUD Coles supermarket gift card at each of their face-to-face appointments (baseline, 6-week and 12-week) in order to assist with changes to their diet which the dietitian may have recommended. This was given in place of the hamper provided to MedDiet participants and was intended to increase dietary compliance and promote attendance. These gift cards were purchased using La Trobe University internal grant funding.

2.8 Data Collection and Outcome Measures

Data collected at each intervention time point have been summarised in **Table 2.3**. Many of the outcomes collected were to be used in the wider MEDINA study, however the data that will be reported in this doctoral research are outlined in bold font. The primary and secondary outcomes, and the clinical outcome measures will be detailed in this chapter. Additional information regarding the overarching MEDINA study is presented in the published protocol paper.[345]

2.8.1 Demographic data

Information regarding participant age, sex, marital status, living arrangements, level of education, employment information, smoking status, ethnicity and languages spoken was collected using a common research form (CRF) created for this trial, **Appendix 3.3.** This information was collected at each participant's baseline (0-week) appointment.

2.8.2 Primary Outcomes; Inflammatory Markers

The primary outcomes reported in this doctoral research are inflammatory cytokines; high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and adipokines; adiponectin, leptin and resistin.

Each participant completed an overnight, 12-hour fast (minimum 10 hours, maximum 14 hours) and fasting blood samples were collected from participants by a trained phlebotomist at the Alfred Hospital Pathology Clinic using Serum Separator Tube (SST) vacutainers, which were then processed and stored by a trained MEDINA researcher. At the Alfred Hospital Gastroenterology Department laboratory, the 8.5mL sample was centrifuged at 2.4 RPM for 10 minutes and the sera separated and frozen at -80°C. Four samples were collected at each timepoint for every participant. Once all enrolled participants had completed the intervention period, all frozen samples were transported on dry ice to Deakin University, Burwood, Melbourne, for further analysis.

Alongside collaborators from Deakin University, Melbourne, the doctoral candidate (AR) assisted in the analysis of cytokine and adipokine markers using milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA). The kits simultaneously measured serum levels of cytokines (interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), cat num: HSTCMAG-28SK) and metabolic hormones (Adiponectin, Resistin and Leptin, cat num: HMHEMAG-34K) as previously described in Gabel et al. (2016).[357] Each assay was performed according to manufacturer's

instructions provided within the kit and all samples were run in duplicate. Briefly, 25µl of the provided standards, controls or blanks were added to the appropriate wells, while 25µl of thawed plasma aliquots were added to the sample wells. 25µl of assay buffer was then added to each well containing the plasma samples, while 25µl of the provided serum matrix was added to wells containing standards, controls and blanks. 25µl of working solution containing multiple microbeads labelled with specific antibodies against each of the aforementioned factors were then added into each well and allowed to incubate overnight on a plate shaker at 4°C. The plate was then washed twice with 200µl Milliplex wash buffer and the beads were incubated for one hour at room temperature in 25 µl of detection antibodies. 25 µl of Streptavidin-Phycoerythrin was then added to each well and allowed to incubate for 30min at room temperature with agitation. The plate was then washed twice more and 150µl of sheath fluid was added to each well and the plate was read on Bioplex 200 multiplex system (Bio-Rad Laboratories, Hercules, CA) and data was analysed using Bioplex manager software (V.6.0, Bio-Rad Laboratories, Hercules, CA).

Mean inter-assay coefficient of variation for the 5 factors analysed were as follows: IL-6: 5.5%; IL-10: 5.1%; TNF-α: 4.7%; Ghrelin: 5.7%; Leptin: 3.7%. Intra-assay coefficient of variation was determined by replicate analysis (n=11) of the provided assay quality controls, the results were as follows: IL-6: 7.8%; IL-10: 7.7%; TNF-α: 6.9%; Ghrelin: 5.9%; Leptin: 4.7%.

hs-CRP was not analysed as per the methodology above, instead it was analysed along with routine biochemistry (details to follow) by the Alfred Pathology Laboratory. All cytokine and adipokine markers were measured at the baseline (0-week) and end intervention (12-week) timepoints for participants in both dietary intervention arms.

2.8.3 Secondary Outcomes

2.8.3.1 Anthropometry, Body Composition and Haemodynamic Measures

Anthropometric data collected included measurement of the participant's height (cm), weight (kg), waist circumference (cm), hip circumference (cm) and neck circumference (cm). Bioelectric Impedance Analysis (BIA) was available within the Alfred Hospital Nutrition Department and was used to measure body composition with the machine brand and model; SECA mBCA 515. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and resting heart rate (bpm) were measured by a trained MEDINA researcher using the Omron Model 705IT machine, standard or large cuff.

Measurement protocol for each anthropometric measure were developed based on the measurement techniques and equipment recommended within the International Standards for Anthropometric Assessment published by the International Society for the Advancement of Kinanthopometry.[358]

Body mass Index (BMI) was calculated using height and weight measurements by dividing weight (kg) by height (m) squared (kg/m^2). Cut offs for BMI were based on the internationally recognised

World Health Organisation guidelines for normal weight, overweight or obese (BMI 18.5-24.9, 25-29.9 or >30kg/m2, respectively).[394] All of the above measurements were collected at each of the face-to-face appointments (baseline (0-weeks), 6-weeks and 12-weeks).

A detailed description of the protocol for each measurement has been outlined below:

Height

- The participants height was measured using a wall-mounted stadiometer.
- If the stadiometer was not wall-mounted, the researcher would place it on a hard level floor.
- The participant was asked to remove their shoes and feet were bare or in stockings.
- The participant was asked to remove hats, hair slides, etc. (if wearing them).
- The participant was directed to stand upright with the heels of their feet, buttocks and occiput (back of skull) touching the backboard of the stadiometer.
- The participant's head was placed in the Frankfurt Plane position. This is the standard plane used for the correct orientation of the head, recognised as a line passing through the tragion (front of the ear) and the lowest point of the eye socket (see **Figure 2.2**).
- The participant was asked to stand tall, relax their shoulders and take a deep breath.
- The measuring arm of the stadiometer was brought down and placed on the top of the participant's head, sitting on top of the hair and pushing down.
- Two measurements were taken in this position to the nearest 0.1cm, the mean of which was used for data recording purposes.

Figure 2.2. *Example of the Frankfurt Plane for measurement of height using a stadiometer.*

Weight

- A centre specific, calibrated scale was used to measure the participant's body weight.
- The participant was asked to remove their shoes, socks and any heavy clothing, and were weighed in light, indoor clothing only.
- The participant was asked to remove money, keys and/or any heavy items from their pockets.

- Measurements were taken after voiding, if possible.
- Body weight was measured to the nearest 0.1kg.
- Body weight was measured using the BIA machine.

Waist Circumference

- A non-stretch tape measure was used to measure waist circumference.
- The participant was in a standing position with the abdomen relaxed, arms at their sides and the feet together and breathing normally when the measurement was taken.
- The participant wore light clothing and the measurement was made directly on the skin, if agreeable to the participant. Waist circumference was not measured if the participant was wearing heavy clothing, a corset or has abdominal oedema.
- Using a pen, a dot was made to identify and mark the iliac crest. The measurement was made from the mid-point between the supra iliac crest and lower ribs margin (**Figure 2.3**).
- Again, using a pen a dot was made to identify and mark the bottom of the ribcage (10th rib). The participant was asked to breathe deeply so that this point can be identified.
- The waist circumference measurement is the mid-point between the bottom of the ribcage and the iliac crest, as illustrated below.

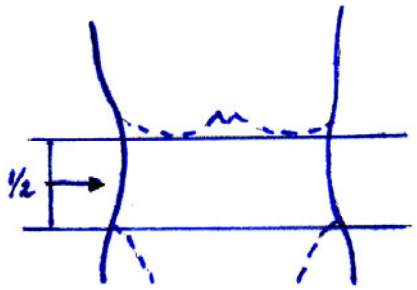


Figure 2.3. *Example of measurement protocol for waist circumference*

- The researcher stands facing the participant and place an elastic tape measure around the subject at the mid-point between the lowest rib and supra iliac crest (defined above) in a horizontal plane.
- The participant was asked to breathe 'normally', and the measurement was taken at the end of the normal expiration, ensuring a true measurement without compressing the skin.
- If the waist was not easily found to take the measurement the researcher asks the participant to bend to the side, it is where the fold is that the waist measurement should be taken.
- The researcher took the measurement whilst standing at the participant's left-hand side.
- Waist circumference measurements were taken in duplicate and to the nearest 0.1cm. The mean value of these two measurements was used as the final value.

Hip Circumference

- The participant stood erect with arms at their sides and feet together, wearing minimal and non-restrictive clothes in the measurement area.
- The researcher squats at the side of the participant and measures from the level of the greater trochanter without compressing the skin. The tape was wrapped horizontally across the participant's hip and placed in the same position on the opposite side of their body (**Figure 2.3**).
- The measurement was recorded in duplicate, to the nearest cm, and the mean of two values was used as the final measurement.

Neck Circumference

- The participant was standing up with their head up and looking straight.
- Using a flexible measuring tape, the researcher measured the distance around the next immediately above the thyroid cartilage (the Adam's apple) (**figure 2.4**).
- The tape was resting on the skin, not held too tightly or loosely.
- The measurement was taken in duplicate and the mean of two measurements calculated as the final value.

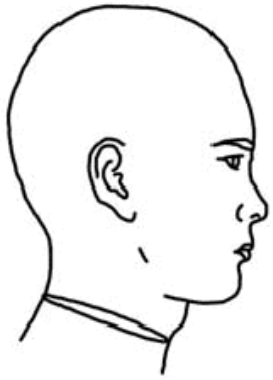


Figure 2.4. *Example of measurement location for neck circumference.*

Haemodynamic Measures

Systolic and diastolic blood pressure (mmHg) and resting heart rate (bpm) were measured at the baseline (0-week) appointment, as well as each subsequent face-to-face visit (6-weeks, 12-weeks, 6-months and 12-months). The blood pressure equipment detailed below were used for all participants in this study:

- Omron Model 705IT Blood Pressure Machine
- Omron standard cuff
- Omron large cuff (if required)

Blood pressure measurements were taken after the participant was weighed, and prior to the measurement of anthropometric measurements. The participant was required to be resting (sitting)

for 5 minutes or longer before the blood pressure measurement was taken using an appropriately sized cuff (pneumatic bag 20% wider than the diameter of the upper arm) on the non-dominant arm supported to heart level. While the measurement was being taken, the participant was asked to remain quiet (not talking or laughing).

Two measurements were taken and recorded. If the readings of either the systolic or diastolic measures differed by more than 10mmHg, additional readings were obtained until values from two consecutive measurements were within 10mmHg. Both readings were recorded and the mean value of the two were used as the final assessment.

Bioelectrical Impedance analysis (BIA) protocol

- The participant was asked to remove their shoes, socks and any metal (keys), wallet, watch or jewellery which were on their person or in their pockets.
- The participant stepped onto the BIA machine and place feet and hands on the correct spots where the BIA electrodes were located.
- The researcher entered the appropriate data into the BIA, including; height and weight (the machine calculates BMI), the participant code, date of birth and activity level.
- Once relevant information was confirmed, the BIA ran the scan.
- The researcher recorded each measurement value (and units) provided by the scan.
- The machine was wiped down with an anti-bacterial wipe after each participant had completed the scan and prior to the next participant being scanned.

2.8.3.2 Biochemistry

Venous, fasting (at least 8h since last eating) blood samples were collected from all participants during face-to-face appointments (baseline, 6-weeks and 12-weeks). The collection and analysis of the blood biomarkers listed in this section were carried out by the Alfred Hospital Pathology. The protocol is described below:

- Fasting glucose and insulin.
- Liver enzymes (total protein, albumin, globulin, bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma- Glutamyl Transferase (GGT)).
- Lipid studies (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides).
- Inflammatory marker (hs-CRP).

The summary of the pathology markers measured with corresponding normal reference ranges are listed in **Table 2.4** (below). Measurement of uncertainty and Clinical Biochemistry Methods adopted by Alfred pathology are described **Appendix 5**. Listed below are the vacutainers collected at each timepoint:

- Baseline: 5ml lithium heparin, 2ml FLOX, 3.5ml SST x3, 3ml EDTA, 6ml EDTA, 8.5ml SST, 4ml CPT x2.
- 6 weeks: 5ml lithium heparin, 2ml FLOX, 8.5ml SST, 6ml EDTA, 8.5ml SST, 42.0ml CPT x2.
- 12 weeks: 5ml lithium heparin, 2ml FLOX, 8.5ml SST, 6ml EDTA, 8.5ml SST, 4ml CPT x2.

2.8.3.3 Insulin Resistance

Insulin resistance is often used as a surrogate marker in NAFLD and T2DM. (refs) In this study insulin resistance was determined using results of insulin and glucose collected from fasting blood samples. The homeostatic model assessment insulin resistance (HOMA-IR) value or score was calculating using the formula: (Glucose (mmol/L) x Insulin (mmol/L))/22.5. refs

2.8.3.4 Liver Outcomes: Intrahepatic Lipids (IHL) and Liver Stiffness Measure (LSM)

Magnetic resonance imaging technique, proton magnetic resonance spectroscopy (^{1}H-MRS), was used to measure hepatic steatosis which was used to calculate IHL (%). Presently, this is the gold standard imaging technique used to quantify hepatic steatosis.[46] In the MEDINA study, all MRI scans were performed using an Avanto 1.5T system (Siemens, Erlangen Germany). One peripheral array and two body array coils obtain coverage from the diaphragm to the pelvis and the standard spine array coil provides posterior coverage. A 3x3x3cm volume of interest was centred within the right lobe of the liver (avoiding major vessels and ducts) and in the right vastus lateralis muscle belly. On a few occasions when body habitus prohibited use of vastus lateralis, the vastus medialis was used for all scans in that individual. All voxel positions were documented and saved on the initial MRI examination to aid reproducibility on subsequent scans. Hepatic and muscle spectra were acquired using the PRESS (point resolved spectroscopy) technique (TR = 3000 ms, TE = 35 ms, 16 measurements, 1024 sample points). Excitation water suppression was used to suppress the water signal during data acquisition. Unsuppressed water spectra were also acquired in vivo for use as the internal standard. Hepatic spectral data were post-processed by magnetic resonance user interface software (jMRUI version 3.0, EU Project). 1H-MRS processing was performed by an experimenter blinded to participants and treatment allocation.

Transient Elastography (TE) ultrasound was carried out by Hepatologist's at the Alfred Hospital using a Fibroscan® machine. TE at 50Hz uses ultrasound to make measurements of the stiffness of the liver. The velocity of a vibration wave (or shear wave) is measured by the time it takes to travel to a particular depth inside the liver.[202] A minimum of 10 valid readings were taken in a single sitting and the median result expressed in kilopascals, which was then interpreted, along with other clinical and biochemical indications, to infer the level of fibrosis. The process requires that the

participant lays on a bed with their right arm raised, whilst a probe similar to an ultrasound probe was placed on their abdomen near the liver. The patient could feel gentle clicks whilst the machine takes the measurements, however it is not painful.[202] Liver stiffness measure was determined and reported in Kilo Pasqual's KPa. Recent Australian consensus guidelines on TE recommend patients fast for two hours prior to the procedure, this was included in the study protocol for this trial.[345]

2.8.4 Questionnaires

Participants completed a number of questionnaires at each timepoint. The questionnaires are listed below with a brief description of their use. All questionnaires were not analysed for this doctorate, the underlined data was derived for this thesis;

- 3-day food diary (**Appendix 6.1**): used to assess habitual dietary intake and dietary compliance. Participants were asked to keep a detailed record of all food and fluid consumed over three days, specifically two weekdays and one weekend day. In order to minimise recall error participants were advised to record their intake throughout the day or at the end of each day. During each face to face appointment (weeks 0, 6 and 12) the study dietitian thoroughly checked the food diary to ensure enough detail was provided and to clarify any errors or misinterpretations while the participant was present. Portion sizes and quantities of food, beverage or ingredients were measured using household measures (e.g., teaspoons, tablespoons, cups, etc.).
- PREDIMED Checklist for the MedDiet (**Appendix 6.2**): uses a 14-point score to calculate a crude measure of adherence to a MedDiet. This tool was developed, validated and used in the large-scale PREDIMED trial. Specifically, the tool has been validated against FFQ-derived data in which the PREDIMED score was inversely associated with 10-year estimated CHD-risk in the Spanish PREDIMED cohort.[359] This MedDiet scoring tool has been widely used and is considered to be a valid and reliable tool.[360]
- PREDIMED Checklist for the LFD (**Appendix 6.3**): uses a 14-point score to calculate a crude measure of adherence to the LFD. This tool was developed, validated and used in the large-scale PREDIMED trial.
- The Cancer Council Food Frequency Questionnaire (FFQ), also known as the Dietary Questionnaire for Epidemiological Studies (DQES): was developed by the Cancer Council Victoria in the 1980s to measure dietary intake of participants in the Melbourne Collaborative Study.[361] The updated FFQ (modified since 1980s version) is designed to be self-administered and covers dietary intake over a period of 12-months.
- Active Australia Questionnaire: used to measure physical activity. This questionnaire is validated and widely considered to be reliable.[362]
- The 36-item Short Form Survey (SF-36): allows participants to evaluate and self-report health determined quality of life. This is a reliable and validated questionnaire.[363]

Table 2.3. Summary of data collected at MEDINA Study appointments during the 12-week intervention

Timepoint	Data Collected	Questionnaires	Tests and Imaging	Biomarkers
Baseline (0-week)	Demographic Data Anthropometry[1] Dietary Consultation & Goal Setting	3-day food diary PREDIMED Checklist FFQ Active Australia SF-36	Buccal swab Pathology samples: - Fasting blood test - PBMC - Urine Imaging: - 1H-MRS - Fibroscan Other: - BIA - DEXA (optional)	Fasting glucose and insulin HbA1C LFTs: - ALT - AST - GGT - ALP Lipid Profile: - TC - LDLC - HDL-C - Triglycerides U&Es, FBE Fe studies Vitamin A, E, D C peptide Inflammatory markers: - hs-CRP - TNF-α - IL-6 - Adiponectin - Leptin - Resistin
Mid-Intervention (6-week)	Anthropometry[1] Dietary Consultation & Goal Setting	3-day food diary PREDIMED Checklist FFQ Active Australia SF-36	Pathology samples: - Fasting blood test - Urine Other: - BIA	Fasting glucose and insulin LFTs: - ALT - AST - GGT - ALP Lipid Profile: - TC - LDLC - HDL-C - Triglycerides
End-Intervention (12-week)	Anthropometry[1] Dietary Consultation & Goal Setting	3-day food diary PREDIMED Checklist FFQ Active Australia SF-36	Pathology samples: - Fasting blood test - PBMC - Urine Imaging: - 1H-MRS - Fibroscan Other: - BIA - DEXA (optional)	Fasting glucose and insulin HbA1C LFTs: - ALT - AST - GGT - ALP Lipid Profile: - TC - LDLC - HDL-C - Triglycerides U&Es, FBE Fe studies Vitamin A, E, D

				C peptide Inflammatory markers: - hs-CRP - TNF-α - IL-6 - Adiponectin - Leptin - Resistin
Follow-up (6-month)	As per mid-intervention appointment			
Follow-up (12-month)	As per end-intervention appointment			
Phone call follow-ups (2w, 4w, 9w)	Goal Setting			

[1]Anthropometry includes weight (kg), height (cm), BMI (kg/m^2), neck circumference (cm), waist circumference (cm), hip circumference (cm), waist-to-hip ratio. Abbreviations: FFQ, food frequency questionnaire; PBMC's, peripheral blood mononuclear cells; BIA, Bioelectrical impendence analysis; DXA, Dual energy x-ray absorptiometry; HbA1c, Haemoglobin A1c; LFT's, Liver Function Test's; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, Gamma-glutamyl-transferase; HDL, High Density Lipoproteins; LDL, Low density Lipoproteins; TG, Triglycerides; U&E's, urea and electrolytes; FBE, full blood examination; Fe, Iron; hs-CRP: high sensitive c-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, Interleukin-6;

Table 2.4. Summary of the pathology markers measured with corresponding normal reference ranges

Biomarker	Collection Method	Technicians	Reference Range
Inflammatory markers			
hs-CRP	Serum Vacutainers: SST Special instructions: A cardiac risk marker, normally only performed on outpatients.	The PhD Candidate with Lab Assistants at Deakin University	Normal: <3.0mg/L Chronic low-grade elevation: 3 - 10mg/L Acute elevation: >10mg/L
TNF-α	Serum Vacutainers: SST		No established reference range.
IL-6	Serum Vacutainers: SST		No established reference range.
Adiponectin	Serum Vacutainers: SST		No established reference range.
Leptin	Serum Vacutainers: SST		No established reference range.
Resistin	Serum Vacutainers: SST		No established reference range.
Insulin resistance			HOMA-IR ≥2.0 mmol/L
Glucose	Plasma and Serum Vacutainers: Fluoride Oxalate, Lithium Heparin, SST Special instructions: Fasting	Alfred Health Pathology	Fasting: 3.5 - 6.0 mmol/L
Insulin	Serum Vacutainers: SST and a Fluoride/Oxalate tube for Glucose must be collected at the same time. Special instructions: Fasting, on ice and to lab immediately.		< 25 mIU/L
Liver Enzymes			
AST	Serum Vacutainers: SST	Alfred Health Pathology	Adult 18+ years: Male: <35 U/L Female: <30 U/L
ALT	Plasma and Serum Vacutainers: SST, Lithium Heparin		Adult 18+ years: Male: < 40 U/L Female: < 35 U/L
GGT	Serum Vacutainers: SST		Male: < 62 U/L Female: < 38 U/L
ALP	Plasma and Serum Vacutainers: SST, Lithium Heparin		Adult >/= 22 years: Male and Female: 30 - 110 U/L

Total Protein	Serum Vacutainers: SST		Adult 19+ years: Serum: 60 - 80 g/L
Albumin	Blood Vacutainers: SST		Adult 19+ years: Serum: 33 - 46 g/L
Globulin	Blood Vacutainers: SST, Lithium Heparin		Male and Female: 24 – 39 g/L
Bilirubin	Plasma and Serum Vacutainers: SST, Lithium Heparin		Male and Female: < 21 umol/L
Lipid Studies			
Total Cholesterol	Serum Vacutainers: SST	Alfred Health Pathology	Recommended: < 5.5 mmol/L
LDL-C	Serum Vacutainers: SST Special instructions: Fasting		Recommended: < 3.5 mmol/L LDL is not measured directly but is calculated using the Friedewald formula: LDLC = (total CHOL)-(HDLC)-(TG/2.2). This formula cannot be used where TG > 4.5mmol/L
HDL-C	Serum Vacutainers: SST Special instructions: Fasting		Recommended: > 1.0 mmol/L
Triglycerides	Serum Vacutainers: SST Special instructions: Fasting		Recommended: < 2.0 mmol/L

Abbreviations: hs-CRP: high sensitive c-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, Interleukin-6; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, Gamma-glutamyl-transferase; HDL, High Density Lipoproteins; LDL, Low density Lipoproteins.

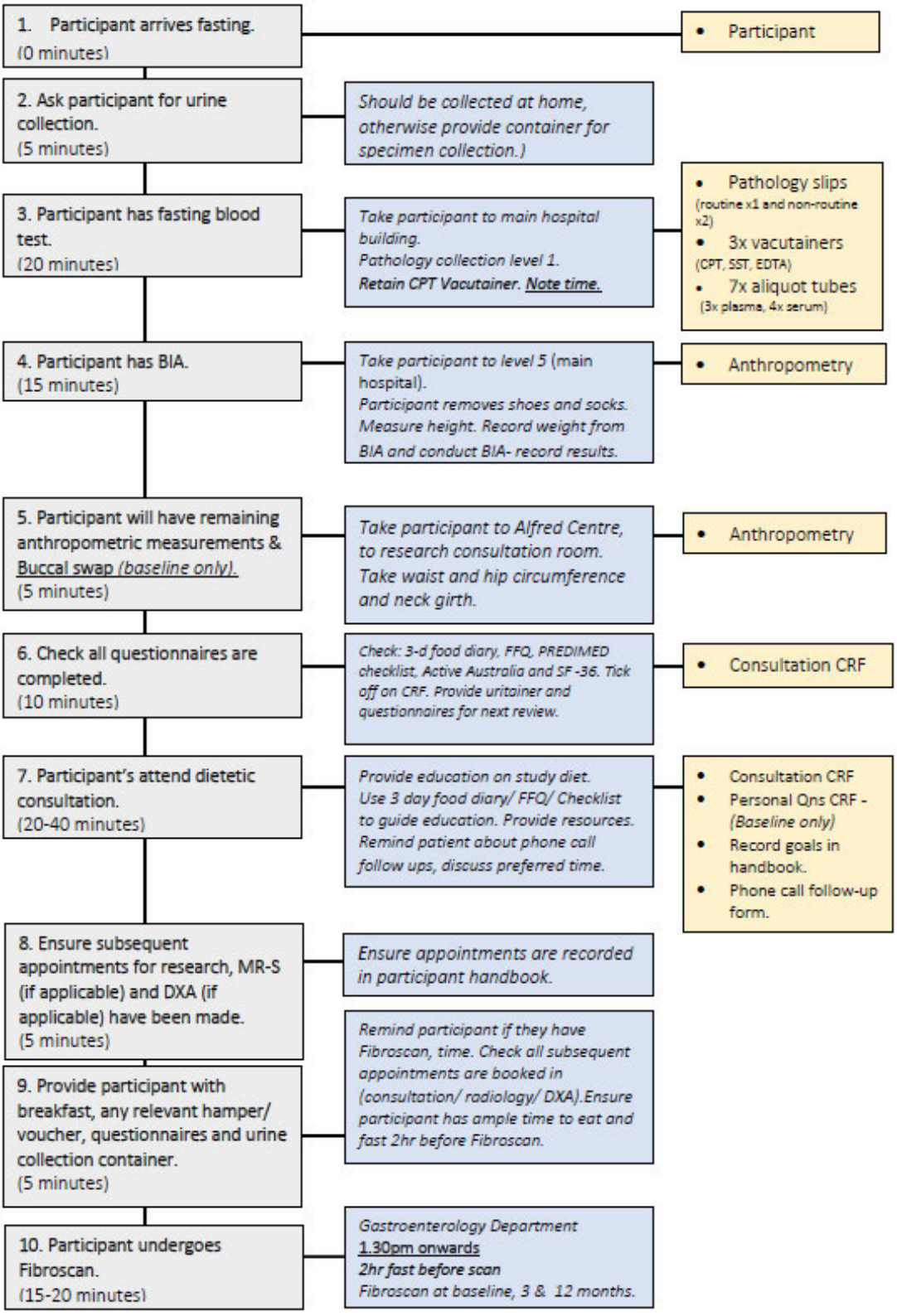


Figure 2.5. Summary of participant face to face appointment at baseline, 6-weeks, and 12-weeks.

Reprinted with permission from George, E. S. (2017). *A Mediterranean diet for the management of non-alcoholic fatty liver disease* (Doctoral thesis, La Trobe University, Melbourne, Australia). Retrieved from http://hdl.handle net/1959.9/564467

2.8.4.1 Dietary Assessment

Three-day food diaries at each timepoint were entered into the software FoodWorks 9™ and analysed using the following databases; AUSNUT 2013, AusBrands 2015 and AusFoods 2015. Diet composition data –macronutrient, micronutrient and food group intake – were exported from FoodWorks 9™ for analysis. Nutrient and food group definitions, classifications, serving sizes were based on the settings in Xyris, Foodworks 9™ software and can be found in **Appendix 7**.

2.8.4.2 Adherence to the Mediterranean Diet

The PREDIMED score was used to measure adherence to a Mediterranean dietary pattern. The PREDIMED score is a 14-point checklist that was developed and validated by researchers in the large-scale PREDIMED trial to assess the effects of a MedDiet in patients with cardiovascular disease (CVD).[364] The scoring criteria is +1 for adherence to a component of the checklist and 0 for non-adherence to a component, thus a higher overall score indicates greater adherence. Each component of the checklist reflects a key component (or principle) of the MedDiet, such as use of olive oil as main culinary fat, and increased consumption of vegetable, fruit, fish, legumes and nut serves. One component focuses on the preferential consumption of white meat over red meat and another component regards the regular consumption of one glass of red wine per day (favourable). Reducing consumption of commercial sweets and using sofrito cooking method (a red sauce with onions and/or garlic and/ or leeks) are also scored favourably on the checklist. An example of the PREDIMED checklist is provided in **Appendix 6.2**. The checklist was completed by all participants in the MedDiet intervention group at baseline (0-weeks), mid-intervention (6-weeks) and end-intervention (12-weeks). To avoid any confusion or errors in reporting, the consulting dietitian reviewed the checklist during the dietary consultation. This ensured accuracy of reporting and the opportunity to cross-check the information on the PREDIMED checklist was consistent with diet data reported in the three-day food diary. Participants in the LFD intervention group did not complete the MedDiet PREDIMED score during the intervention, however the checklist was completed retrospectively for participants in the LFD group using three-day food diaries so that comparisons with MedDiet adherence could be made between groups. This was done by a trained MEDINA researcher (AR).

2.8.4.3 Adherence to the Low Fat Diet

A separate 9-item PREDIMED checklist was used as a crude measure for adherence to a LFD in the LFD group. This checklist was also developed by the aforementioned PREDIMED Study and contained key components of the LFD and questions regarding low-fat cooking methods, for which participants scored +1 point for adherence and '0' for non-adherence. The greater the score (up to 9), implied the greater adherence to the LFD. An example of this checklist is presented in **Appendix 6.3**. Each participant in the LFD group completed this checklist prior to each face-to-face

appointment (0-, 6- and 12-weeks) and the checklist as reviewed by the consulting dietitian during their dietary consult to ensure participants understood the checklist and to minimise errors or inconsistencies.

2.8.5 Dietary Inflammatory Index

The DII® score was calculated for each participant at baseline and at the end of intervention (12-week) to provide a measure of the theoretical inflammatory potential of their diet. As a reference for each diet arm, the DII was also calculated based on the MedDiet intervention 2-week meal plan and the LFD intervention 2-week meal plan.[365] The meal plans were entered into FoodWorks8 and additional nutrient databases as necessary, and a complete list of the 45 nutrient parameters listed in the index (**Appendix 2**) were exported into an excel spread sheet which was used to calculate the DII score of each diet plan. Researchers of the AUSMED Study, a study of the MedDiet vs Low-Fat Diet in Australian patients who have experienced a coronary event, then sent this spreadsheet electronically to collaborators Dr Nitin Shivappa and Prof James Hebert of the University of South Carolina, USA, who performed calculation of the DII scores. This data has been published elsewhere.[338] The AUSMED Study and MEDINA Trial were conceptualised by the same research group at La Trobe University, Melbourne, Australia, and both trials utilise the same dietary intervention profiles. Therefore, the DII scores of each meal plan were used in this study to analyse if the inflammatory potential of the MedDiet meal plan differed to that of the LFD meal plan, and if participants in respective groups increased adherence to their respective diets in turn improving their DII score.

Dietary data for intake of energy and nutrients (total fat, saturated fat, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), omega 3, omega 6 (linoleic acid only), trans fat, cholesterol, carbohydrate, protein, vitamin B12, vitamin B6, vitamin A, vitamin C, vitamin E, caffeine, alcohol, fibre, beta carotene, folic acid, iron, magnesium, niacin, riboflavin, selenium, thiamine and zinc) were determined through the standard FoodWorks9 nutrient analysis software using AUSNUT 2013, AusBrands 2015 and AusFoods 2015 databases which was collected and input from the 3-day food diaries collected at each timepoint. Food and nutrient parameters that were not available from FoodWorks9 and therefore not calculated for participants in this cohort, were: garlic, ginger, saffron, turmeric, vitamin D, green/black tea, flavan-3-ol, flavones, flavanols, flavanones, eugenol, anthocyanidins, isoflavones, pepper, thyme, oregano and rosemary. The units of all nutrient components were converted to those prescribed in the index, in the case that they were not already in that measurement unit. Once the data for the 28 food and nutrient parameters was calculated using FoodWorks, data was collated in a Microsoft Excel spread sheet for each timepoint (baseline and end-intervention). An electronic copy of the spreadsheet was then provided to collaborators Dr Nitin Shivappa and Prof James Hebert of the University of South Carolina, USA, who calculated the DII scores of each participant in the MEDINA Study.

2.9 Genotyping

Cheek cells were collected using buccal swabs (Isohelix Swabs SK1S); and DNA was extracted using Wizard Genomic DNA Purification Kit (Promega,USA) catalogue number A1120 according to manufacturer's protocol. Extracted DNA samples were stored at -20^{o}C in micro-centrifuge tubes until further analysis. The extracted DNA was quantified and checked for purity by Nanodrop spectrophotometer. Thereafter the DNA samples were genotyped on a SNP array using quantitative real-time PCR on a Life Technologies QuantStudio 12K Real Time PCR system using the cycle relative threshold (Crt) method. The reactions were carried out based on two assays, each with two primers and a Taqman probe, one specific to the target SNP. The output from this system is then entered into and analyzed by Life Technologies Copy Caller software (v 2.1).

2.10 Ethics Approval

Participant appointments were conducted at the Alfred Hospital, Melbourne, and a full high-risk ethics application was submitted and approved by Alfred Health (project number: 76/14). Low risk ethics applications were submitted and approved from Eastern Health and Melbourne Health where screening and recruitment was conducted. Expedited human research ethics committee review was approved from La Trobe University which was the overseeing institution for the clinical trial. Ethics was also obtained from St Vincent's Hospital however there were no participants included from this site within this doctoral thesis. Approval certificates are available in **Appendix 8**.

2.11 Sample Size

A power calculation for the overarching MEDINA trial was performed by a trained statistician based on IHL (%) as the primary outcome. The sample size was calculated based on data previously published by Ryan et al. (2013) with the assumption that the MedDiet group would achieve 25% change in IHL and the LFD group would achieve 5% change in IHL.[295] To detect this 20% difference in change of IHL between groups, with at least 80% power (type I error = 5%), the trial needed to recruit 17 participants to each study group. After adjustment for 20% dropout rate, the required sample size was calculated to be 17/0.8 = 21 participants per group.

The candidates systematic literature review [256] provided insight into the limited number of dietary intervention trials which have investigated the effects of diet on inflammatory markers in patients with NAFLD. From the data available for the primary outcome measures of this thesis, cytokines; hs-CRP, TNF-α and IL-6, and adipokines; adiponectin, leptin and resistin, a sample size calculation was performed prior to analysis of these outcomes. An *a priori* power analysis powered to see a significant change in each cytokine and adipokine marker was based on the statistical test for 'Means: Difference between two independent means (two groups)'. The sample size calculation was performed using the statistical software program G*Power 3.0.10. The calculation included the

effect size, power (1-β err prob) of 80% and $\alpha<0.05$. The estimated sample size prediction required between 120 – 350 participants, depending on the sensitivity of each inflammatory marker. These calculations are available in **Appendix 9**. Recruitment of a sample size this large was not achievable due to study funding or feasible in keeping with PhD timelines, therefore it was deemed more appropriate to use the data obtained from this study as pilot and feasibility trial data, to add to the limited evidence in this area and to inform future study design, recruitment and data analysis. Additionally, this was a secondary analysis and is largely exploratory research. The outcomes measured in chapters 5 and 6 of this thesis are largely exploratory in nature, hence formal sample size calculations were not performed. Information is provided in the relevant chapters' methods sections.

2.12 Data Analysis

Details regarding the statistical analysis conducted for each research chapter are described in the respective methods sections of each chapter. All statistical analyses were performed using SPSS statistical package version 25.0 (IBM Corp, Released, 2017).

2.13 Investigator Involvement in Research Tasks

Table 2.5 (below) provides an overview of the various research tasks carried out by the Candidate and other investigators as part of the MEDINA Study. The Candidate substantially contributed to all stages of trial coordination, screening and recruitment of participants, data collection, entry and analysis.

Table 2.5. Overview of research tasks related to this thesis and involvement of the Candidate and other investigators

Research Task	Researcher/Investigator	Location
Study Design		
Trial Protocol	Dr Elena George Prof Stuart Roberts A/Prof Amanda Nicoll Dr Marno Ryan Dr Agus Salim Prof Catherine Itsiopoulos Dr Audrey Tierney (PI)	La Trobe University The Alfred Hospital, Melbourne Eastern Health St Vincent's Hospital, Melbourne
Diet Interventions	Dr Elena George Teagan Kucianski Prof Catherine Itsiopoulos Dr Audrey Tierney	La Trobe University
Data Collection		

Screening and Recruitment	**Anj Reddy (Candidate)** Dr Elena George Tonya Paris	The Alfred Hospital, Melbourne St Vincent's Hospital, Melbourne The Royal Melbourne Hospital
Randomisation	Dr Elena George	La Trobe University
Mediterranean Diet Dietitian	Dr Elena George Lauren Manning	La Trobe University
Low-Fat Diet Dietitian	Lisa Murnane Katie McKean	The Alfred Hospital, Melbourne
Provision and collection of study hampers, forms and questionnaires during face-to-face appointments (weeks 0, 6 and 12)	**Anj Reddy (Candidate)** Dr Elena George	The Alfred Hospital, Melbourne
Anthropometry and Body Composition (via bioelectrical impedance analysis)	**Anj Reddy (Candidate)** Dr Elena George	The Alfred Hospital, Melbourne
Blood pressure and heart rate	**Anj Reddy (Candidate)** Dr Elena George	The Alfred Hospital, Melbourne
Dual Energy X-Ray Absorptiometry scans	**Anj Reddy (Candidate)** Dr Elena George	La Trobe University
Radiology (H1 MRS)	Baker Heart and Diabetes Institute Radiologist	Baker Heart and Diabetes Institute, The Alfred Hospital Melbourne
Transient Elastography (Fibroscan®)	Prof Stuart Roberts Dr William Kemp Dr Matthew Kitson	Department of Gastroenterology, The Alfred Hospital Melbourne
Data entry	**Anj Reddy (Candidate)** Dr Elena George Tonya Paris Stacey Anne Fong-To Lauren Manning	La Trobe University
Data Analysis		
Demographics	**Anj Reddy (Candidate)**	
Food diaries/ FoodWorks analysis	**Anj Reddy (Candidate)**	
Dietary Inflammatory Index	**Anj Reddy (Candidate)** Dr Elena George Dr Nitin Shivappa Prof James Hébert	La Trobe University University of South Carolina
Single Nucleotide Polymorphisms	**Anj Reddy (Candidate)** Dr Chee Kai Chan	La Trobe University Fitgenes©
Laboratory Analysis		

Blood Biomarkers	Alfred Health Pathology laboratory technicians	The Alfred Hospital, Melbourne
Inflammatory Markers	**Anj Reddy (Candidate)** Dr Shaun Mason Dr Paul Della Gatta	Deakin University, Melbourne
Genotyping	Dr Chee Kai Chan	Fitgenes©

3 Sex and metabolic differences in patients with NAFLD: The MEDINA Study Cohort at Baseline

3.1 Abstract

Introduction: Non-Alcoholic Fatty Liver Disease is the most prevalent liver disease worldwide and continues to rise in parallel with rates of obesity and type 2 diabetes mellitus. Up to 20% of people with the disease will progress to more advanced stages of non-alcoholic steatohepatitis and progressed liver disease. Insulin resistance and inflammation are central underlying features of non-alcoholic fatty liver disease and drive disease progression if left unmanaged with dietary and lifestyle modification. This chapter describes the demographics of participants with biopsy or ultrasound proven non-alcoholic fatty liver disease, and assesses anthropometric, body composition, biochemical, inflammatory, liver and dietary characteristics of the group, and explores sex differences and differences between those with and without metabolic syndrome.

Methods: This was a cross-sectional analysis of forty-two participants with non-alcoholic fatty liver disease recruited to participate in the Mediterranean Dietary Intervention for Adults with Non-Alcoholic Fatty Liver Disease (MEDINA) Study. Participants were recruited from liver outpatient clinics at three hospitals in Melbourne; the Alfred Hospital, Eastern Health and the Royal Melbourne Hospital. Medical records were screened, and eligible participants attended a baseline appointment where self-reported sociodemographic data and 3-day food diaries were collected. Anthropometry, body composition, biochemical samples and liver outcomes were measured.

Results: Enrolled participants (60% female, mean age 52 ± 13 years) were mostly obese (mean BMI 32 ± 6 kg/m^2) and insulin resistant (mean HOMA-IR 3.8 ± 2.9). Over half the group met criteria for metabolic syndrome but did not have a worse metabolic or inflammatory profile than those who did not. Females tended to be older ($p < 0.05$), more insulin resistant and have higher leptin ($p < 0.05$) and IL-6 concentrations. Males had lower (more unfavourable) adiponectin levels and tended to have higher visceral fat content ($p < 0.05$). This cohort were consuming less than the population requirements for energy and carbohydrate intake set by NHMRC guidelines to reduce chronic disease risk, though exceeding recommended intakes of total and saturated fat, total sugars, and protein. They were also not meeting the required serves of fruits and vegetables.

Conclusion: Non-alcoholic fatty liver disease is a complex disorder. Noting key sex differences such as adipose tissue distribution, levels of insulin resistance and inflammatory status may be useful for screening at the asymptomatic stage. Improvements in diet quality would be beneficial in this group, particularly adhering to dietary and lifestyle guidelines for the management of non-alcoholic fatty liver disease.

3.2 Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is characterised by fatty infiltration of the liver that occurs in the absence of excess alcohol consumption, which can progress to a broad spectrum of liver disease including Non-Alcoholic Steatohepatitis (NASH), with various degrees of lobular inflammation, fibrosis and cirrhosis.[366] Once cirrhotic, individuals are at increased risk of developing hepatocellular carcinoma, end-stage liver disease and all-cause mortality.[367] NAFLD in its simplest form however, has been identified as an independent risk-factor for cardiovascular disease which is also the leading cause of death in this population.[368, 369] It is also projected to become the leading indication for liver transplantation in Western countries by 2030.[370] NAFLD is estimated to be prevalent in up to 30% of the population world-wide, of whom approximately 10-20% will develop NASH and 9-15% of NASH patients will progress to cirrhosis.[371] The growing clinical burden of NAFLD has been evident over a number of decades, though the scope of the disease was confined to liver-related morbidity and mortality. In more recent years, due to strong association and epidemiological studies fatty liver is increasingly seen as a multisystem disease which affects several extra-hepatic organs and regulatory pathways.[370]

Obesity is a common characteristic and risk factor for individuals with chronic metabolic diseases. The overlap between obesity, type 2 diabetes mellitus (T2DM) and the metabolic syndrome (MetS), as well as dyslipidaemia is widely reported in the literature. An estimated 70-80% of obese individuals have NAFLD, and at least 15-20% have NASH.[372] Similarly, up to 70% of patients with T2DM also have NAFLD, 88% of patients with NASH have the MetS, and dyslipidaemia is present in 50-60% of individuals with NAFLD.[373, 374] Obesity is caused by excess energy intake, usually coupled with physical inactivity, leading to excessive accumulation of adipose tissue. The storage and expansion of excess adipose tissue is associated with an imbalance in glucose and lipid metabolism and increases secretion of pro-inflammatory markers.[370] Insulin resistance (IR) increases metabolic stress and lipolysis,[373] and the dysregulation of non-esterified fatty acids which are taken up by the liver, skeletal muscle and pancreas.[375, 376] Hyperinsulinemia is strongly associated with the prevalence of NAFLD, as it develops to facilitate normal glucose homeostasis, and drives *de novo* lipogenesis through upregulation of the transcription factor Sterol Regulatory Element Binding Protein-1c (SREBP-1c).[122, 376]

The progression of NAFLD to NASH is poorly understood, but is thought to be driven by a more progressive, inflammatory disease phenotype, suggesting that there are many "hits" acting on the liver in parallel.[377] Tilg and Moschen (2010) proposed a "multiple hit hypothesis" which includes IR, gut and adipose tissue derived hormones, nutritional, genetic and epigenetic influences as the main contributors to disease progression.[377] Among this cascade of mechanisms, key inflammatory cytokines; C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and adipokines; adiponectin, resistin and leptin, are recognised as biomarkers of chronic low grade inflammation in NAFLD.[377] Adiponectin is a classic adipokine associated with NAFLD, acting as

an anti-inflammatory and anti-fibrotic mediator in the development and progression of the disease. Unlike other inflammatory markers, adiponectin is suggested to be found in lower concentrations in those with NAFLD and NASH. Cross-sectional studies have reported associations between elevated serum TNF-α and IL-6 with NAFLD, while the relationship between resistin and leptin with NAFLD is less often reported. Some clinical trials have investigated the effect of dietary interventions on liver histology (including severity of hepatic steatosis) and inflammatory markers finding that dietary modification can improve both liver and inflammatory outcomes,[255, 378-381] though one specific diet has not been found superior over others. Furthermore, the degree to which inflammatory markers influence liver histology, hepatic steatosis and progression to a more severe disease phenotype has not been explained. Individuals with NAFLD who are attending the clinical setting for care/treatment are ideal for recruitment into experimental studies and results could form the basis for more targeted dietary treatment approaches.

Indeed, visceral adiposity and elevated circulating inflammatory markers are independent risk factors for NAFLD. Many studies have found that NAFLD and NASH are associated with visceral adiposity, elevated inflammatory biomarkers and risk of developing cardiovascular disease (CVD),[158, 159, 162, 204, 373, 382] implying NAFLD/NASH as an additional feature of the MetS.[383] A study in both obese and nonobese subjects found a positive correlation between the severity of fatty liver with visceral fat accumulation and insulin resistance, suggesting that visceral fat accumulation may influence hepatic fat infiltration in non-alcoholic fatty liver disease regardless of body mass index.[383] The influence of visceral adiposity as a strong predictor of cardiometabolic risk, chronic inflammation and hypoadiponectinemia was reported in biopsy-proven NASH patients.[384] Interestingly, this study found that compared to healthy controls with comparable values of visceral adiposity NASH patients were more insulin resistant and had remarkably higher hs-CRP and lower adiponectin, implying that NASH predicts a more atherogenic and inflammatory risk profile independent of visceral adiposity.[384] It remains unclear whether the accumulation of adipose tissue and consequent inflammation is a determinant of who develops NAFLD/NASH or whether fatty infiltration of the liver derives inflammatory markers causing adipose tissue inflammation, and therefore systemic inflammation, T2DM and CVD.[383, 385]

Classic risk factors for NAFLD are age, sex and ethnicity. The prevalence of NAFLD is different between males and females, although reports of sex distribution are somewhat inconsistent. Currently, the prevalence of NAFLD is estimated to be approximately 30-40% in males and 15-20% in females.[386] Originally it was thought that NAFLD was more common in females, particularly middle-aged and older women, though these findings were not population based and were subject to potential bias.[387] Data from NHANES III, showed most studies reported that NAFLD was significantly more prevalent in males than in females.[60, 388-390] In males, prevalence of NAFLD and histological NASH were associated with elevated aminotransferase levels (ALT), hepatic fibrosis and mortality.[391] More recently, data gathered from NAFLD-related hospitalisations

in the United States reported a higher proportion of females (61%) than males (39%) presented in a hospital setting.[392] Given these inconsistencies, it remains uncertain whether NAFLD is more prevalent in males or females. The tendency of NAFLD to be influenced by sex is also unclear and mechanisms contributing to the difference, while somewhat defined, remain unclear and under-studied. Some studies attribute higher NAFLD prevalence in males to higher waist-to-hip circumference ratio (WHR) related to higher visceral adiposity and therefore higher hepatic and peripheral IR.[387, 393] Moderate alcohol intake in Caucasian men has been associated with increased hepatic steatosis compared to female counterparts (42% vs 20%, P = 0.03).[386] More commonly reported however, are lifestyle and sex hormone influences in the development and progression of NAFLD – usually impacting the female population. The prevalence of NAFLD in women tends to be higher after menopause.[394] Most of the evidence suggests that oestrogen protects from NAFLD and hormone replacement therapy following menopause can significantly reduce ALT in women with T2DM.[394] The influence of sex is an important factor in the development of chronic conditions such as the MetS and NAFLD and its association with pathological features of inflammation as a risk factor of disease requires investigation.

It has been reported that a NAFLD population generally consume a high-calorie diet consisting of excess saturated fats, refined carbohydrates, high fructose containing beverages and sugar sweetened food items.[395] Omega-3 fatty acids and antioxidants are consumed in lesser amounts by individuals with NAFLD. Highly processed, sweetened foods and high-fructose containing drinks are strongly associated with severity of liver fibrosis in NAFLD.[396] Particularly, fructose consumption is detrimental to the inflammatory pathways and cellular stress pathways.[397] More recently, research on sugar deems glucose and added sugars, not only fructose, to be responsible for hepatic fat accumulation and weight gain.[398] The quantity and quality of dietary fat consumption is particularly important in NAFLD, as the accumulation of intrahepatic and visceral fat is dependent on the ratio of saturated to polyunsaturated fats.[399] Accumulation of visceral fat and adipocyte proliferation further promotes the release and circulation of harmful inflammatory cytokine and adipokine markers.[377] The relationship between diet, inflammation and the development of NAFLD is complex and should be considered as such when considering an appropriate treatment approach.

The MetS is highly prevalent among individuals with NAFLD and both are known risk factors in the development of future CVD events. Considering that NAFLD and the MetS stem from chronic low-grade inflammation and share similar features of metabolic abnormalities, this study intended to explore whether there was added risk of inflammation, metabolic and clinical features in individuals with both NAFLD and the MetS compared to individuals with NAFLD who did not meet criteria for the MetS. The aim of this research chapter was to describe the demographic of individuals with NAFLD who were recruited for the MEDINA trial, at baseline, and to assess anthropometry, body composition, biochemistry, inflammatory markers, liver outcomes and dietary

intake characteristics between males and females and between individuals meeting the criteria for MetS and those that do not have the MetS. The secondary aims of this chapter were to identify associations between inflammatory markers with anthropometry, biochemistry, liver outcomes and dietary intake, to explore predictors of inflammatory markers in this population.

3.3 Methods

3.3.1 Study Design

This study is a cross-sectional analysis of data collected at a baseline (0 week) appointment of individuals with NAFLD, entering the MEDINA trial. The full methodology of the MEDINA study is in accordance with the published MEDINA study protocol.[345] For additional details pertaining to recruitment, study design, outcome measures and data collection, please refer to **Chapter 2** of this thesis. The study protocol was approved by the Human Research Ethics Committee at La Trobe University and at the recruitment and study sites, Alfred Health (76/14), Eastern Health (LR31/2015) and the Royal Melbourne Hospital (HREC/15/MH/268)**(Appendix 8)**.

3.3.2 Eligibility criteria

A detailed outline of the inclusion and exclusion criteria applied for recruitment of participants are outlined in **Chapter 2** (**Section 2.4, Table 2.1**). Briefly, adults (>18 years) who had been diagnosed with NAFLD and/or NASH through ultrasound and/or biopsy within the previous 12-months were eligible to take part in the study. Individuals were required to have a body mass index (BMI) between 20-40 kg/m^2, an elevated serum alanine aminotransferase (ALT) level (>20U/L for females and >30U/L or males), no evidence of any other form of liver disease and no current or past history of cardiovascular, cerebrovascular or peripheral vascular disease. Individuals who could not provide informed consent, were non-English speaking or consumed above the average recommended level (>140g) of alcohol intake per week were excluded from the study. Participants who experienced >5kg weight change within 3-months of screening or were adhering to a commercial diet or consuming hepatotoxic medication, fish or krill oil, or vitamin E, vitamin C or high dose vitamin D were also excluded.

3.3.3 Screening and Recruitment

Patients who attended the liver outpatient clinics at the Alfred Hospital, Eastern Health and the Royal Melbourne Hospital, and who met the eligibility criteria for the MEDINA study were identified through patient appointment lists prior to each liver clinic. Once deemed eligible based on their medical history and clinical results, the patient was then approached by a trained researcher – either in person or over the telephone – and informed of the study, allowing the patient to ask questions and express their interest. If the patient was interested in participating, they were required

to undergo a screening questionnaire designed to further confirm eligibility and sign a participant information consent form (PICF)**(Appendix 3.1)**.

3.3.4 Data collection and outcome measures assessed

3.3.4.1 Demographics

Information regarding participant age, sex and co-morbidities were recorded during the pre-baseline screening questionnaires and correspondence. Further information regarding the diagnosis of co-morbidities or pre-baseline results were extracted from recruitment sites' clinical databases or patient medical histories. Details of ethnicity, smoking status, marital status, living arrangements, education level, employment status and occupation were collected during the baseline appointment using a personal information questionnaire implemented by a trained researcher.

3.3.4.2 Anthropometry and Body Composition

Full details of the anthropometric and body composition measurement procedures are presented in **Chapter 2** (**Section 2.8.3.1**). In brief, measures of neck circumference (NC), waist circumference (WC) and hip circumference (HC) were taken by a trained researcher using a body tape measure during the baseline appointment. To reduce the incidence of error two values were taken for each measurement to the closest 0.1cm, and the average was recorded as the final value. Anthropometric measurements were taken by the same investigator for each participant throughout the duration of the study, where practically feasible. Height was measured using a wall-mounted stadiometer. The participant was asked to remove their shoes and to stand upright with heels, buttocks, and occiput touching the backboard of the stadiometer. Two measurements of height were taken to the nearest 0.1cm, the mean of which was recorded.

Weight and body composition measurements were measured using Bio-Electrical Impedance Analysis (BIA) with the seca® mBCA 515. A trained researcher ensured that the participant had removed shoes, socks and any wallet, keys, watches or jewellery they were wearing or carrying in pockets prior to analysis. Body mass index (BMI) was calculated by the BIA by dividing weight (kg) by height (m) squared, and this value was recorded into the appropriate anthropometry case report form (CRF). BMI cut-offs were based on the internationally recognised World Health Organisation (WHO) guidelines[400] and defined as normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) or obese (>30 kg/m^2). Body composition estimates based on data from BIA analyses in the National Health and Nutrition Examination Survey (NHANES III)[401] were used to define "normal" means and standard deviations for body composition measures including fat mass (FM), fat-free mass (FFM), fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle mass and visceral fat (VF).

3.3.4.3 Blood Pressure and Heart Rate

Systolic and diastolic blood pressure (mmHg) and heart rate (bpm) measurements were taken by a trained researcher using an automated blood pressure monitor (Omron Model 705IT) using a standard or large cuff after the participant had been sitting for five minutes. Two blood pressure measurements were performed on the same non-dominant arm supported to heart level and recorded. If either the systolic or diastolic readings differed by more than 10mmHg, additional readings were obtained until values from two consecutive measurements were within 10mmHg. The mean of the closest two measurements was recorded as the final result.

3.3.4.4 Biochemistry

All participant blood samples were collected by a trained phlebotomist at the Alfred Pathology Service (Alfred Hospital, Melbourne) and analysed at the Alfred Pathology Laboratory. Each participant provided a fasting blood sample at the baseline appointment. Full details of both the Alfred Hospital and MEDINA laboratory analysis protocols are presented in **Chapter 2** (**Section 2.8.3.2** and **Table 2.4**). Along with blood biomarkers analysed by the Alfred Pathology Laboratory, analysis of inflammatory marker hs-CRP was performed by Alfred Pathology Staff. Participants with a measurement of hs-CRP >10mg/L were excluded from analyses (n=3) of this marker as this indicates an acute inflammatory state rather than chronic inflammation. In addition to the samples provided to the Alfred Pathology Laboratory, a trained researcher retained four tubes; 1x 6mL EDTA, 1x 6mL SST and 2x 4mL CPT vacutainers, to be processed and frozen at -80ºC in the Gastroenterology Department of the Alfred Hospital.

3.3.4.5 Laboratory analysis

Samples obtained using SST vacutainers, processed and frozen were used to measure levels of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), adiponectin, leptin and resistin by a trained laboratory technician and MEDINA research personnel (at Deakin University, Melbourne). Milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA) were used to simultaneously measure serum levels of cytokines and metabolic hormones as previously described in **Chapter 2** (**Section 2.8.3.2**). For details of the full methods used to run the assay, mean inter- and intra-assay coefficient of variation values and details of the analysis software, refer to **Chapter 2** (**Section 2.8.2**).

3.3.4.6 Liver Outcomes

In order to quantify intrahepatic lipid (IHL) content, participants underwent magnetic resonance spectroscopy (^{1}H-MRS) with a qualified radiologist at the Baker IDI Heart and Diabetes Institute. All ^{1}H-MRS studies were performed on an Avanto 1.5T system (Siemens, Erlangen Germany).

Participant data was de-identified and hepatic spectral data was post-processed using magnetic resonance user interface software (jMRUI version 3.0, EU Project) by an experimenter who was blinded to participant and treatment allocation.

Liver stiffness, fibrosis and cirrhosis was assessed using transient elastography (TE) in the form of Fibroscan®. The scan was performed and analysed by a liver specialist (Hepatologist or Gastroenterologist) in the Gastroenterology Department of the Alfred Hospital.

3.3.4.7 Dietary Intake

Participants were required to complete a 3-day food diary prior to their face-to face appointment, which would adequately reflect all food and drink items consumed throughout two weekdays and one weekend day during that week. During the dietary consultation, a dietitian reviewed the participant food diary to ensure sufficient detail was provided and no food items had been omitted. Any measurement or item inconsistencies were checked and corrected during this review.

The food diaries were entered into FoodWorks8® software (Xyris Australia Pty Ltd). Databases *AUSNUT 2013, AusBrands 2015* and *AusFoods 2015* were selected for nutrient and food group intake analyses. Data extracted and analysed from FoodWorks 8 includes macronutrient, micronutrient and food group intake.

3.3.5 Metabolic Syndrome Diagnosis

The National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATP III) criteria[402] were used to classify participants in the MEDINA study population as having the MetS if they had three or more of the following criteria at baseline:

- Hypertension: defined by blood pressure ≥130/85 mmHg and/or patients were receiving blood pressure lowering drugs
- Fasting plasma glucose (≥6.1 mmol/L) or patients taking glucose lowering drugs
- Hypertriglyceridemia: defined by fasting plasma triglycerides ≥1.69 mmol/L
- Low HDL-cholesterol: defined by fasting HDL-cholesterol <1.04 for males or <1.29 mmol for females
- Central obesity: defined by waist circumference >88cm for females or >102cm for males.

3.3.6 Statistical Analysis

All statistical analyses were conducted using SPSS® statistical package version 25 (IBM Corp,

Released 2015). Statistical significance was set at $p<0.05$. Descriptive statistics were carried out on a wide range of variables and frequency tables, mean and standard deviation (SD) were generated in order to produce summary tables for participant demographics.

Normality tests were undertaken to assess whether data was normally distributed. Skewness, kurtosis and outliers were checked, and the Kolmogorov-Smirnov statistic was used to assess normality. Normally distributed variables are reported as mean (SD). Non-parametric variables are expressed as median (interquartile range (IQR)). For univariate comparisons between groups, independent samples t-tests were used for parametric data and Mann-Whitney U Tests were used for non-parametric data.

At baseline, anthropometry, biochemistry, inflammatory biomarkers, liver outcomes, and nutrient and food group intake were analysed for the total cohort and then split to identify any differences between sex (males vs. females) and diagnosis of the MetS (individuals with the MetS vs. individuals without the MetS). This was done in an attempt to explore and contrast the characteristics of those deemed metabolically at-risk and assess whether or not they also had less favourable clinical features and liver profile, considering that NAFLD is termed the hepatic manifestation of the MetS. This allows for meaningful comparisons to be made to add to existing literature with sex differences and severity of NAFLD, and to explore characteristics of the participants with the MetS and NAFLD.

Bivariate correlation analyses were used to determine the strength and direction of the relationship between each inflammatory marker (cytokine and adipokine markers) with anthropometric and biochemical variables, as well as nutrient and food group intake variables. Depending on whether each variable was parametric or non-parametric, Pearson and Spearman's rho bivariate correlation analyses were performed accordingly. R-values were reported for strength and direction of the relationship and P-values represent significance of the relationship. R-values were classified as weak (0.10 to 0.29), moderate (0.30 to 0.49) or strong (0.50 to 1.00) correlations.

Multiple linear regression analyses were used to generate prediction equations for all of the significantly ($p <0.05$) correlated variables with each inflammatory marker. For each regression model, non-parametric variables; hs-CRP, TNF-α, IL-6, adiponectin, leptin, energy, total dietary fat and cholesterol intake, polyunsaturated fat and saturated fat (as % of total energy intake), AST, ALT, HOMA-IR and linoleic acid, were included as log-transformed variables in order to conform to normality. Reciprocal transformation was used to normalise the variable glucose, as this variable did not conform to normality using log-transformation. In each regression model, the inflammatory marker was input as the dependent variable and independent variables were grouped together: anthropometric variables, biochemical or dietary intake variables and input as separate steps or "levels" which would explain the added variance within each step. Several stepwise regression models were built by adding or removing predictor variables into a previous model at each step;

later models always included the previous steps, which allowed significant improvement to be identified with each newly added variable. Age, sex and diabetes status were adjusted for in each regression model. Diabetes status was controlled for due to the significant difference in insulin and HOMA-IR between those with diabetes and those without diabetes which has scope to influence the predictive ability of anthropometric, biochemical and dietary variables for NAFLD. Age and sex were considered potential confounders and were controlled for in each model. Before interpreting the results, a number of assumptions were tested, and checks were performed. Assessment of the normal probability plot of standardised residuals and the scatterplot of standardised residuals against predicted values indicated that the assumptions of normality, linearity and homoscedasticity were met. If two variables were similar in nature or one variable encompassed another (for example, insulin and HOMA-IR), only the variable with the strongest correlation was included in the regression model ensuring that multicollinearity would not interfere with the ability to interpret the outcome. Potential violations of the assumption of multicollinearity was assessed by collinearity diagnostic values for Tolerance and variance inflation factor (VIF). Standardised beta (β) regression coefficients squared multiple correlation (R^2) or squared-change correlation (ΔR^2), regression and residual degrees of freedom (df1 and df2, respectively) and significance level (P-value) were reported for each model.

3.4 Results

3.4.1 Demographics of the NAFLD cohort

Participant demographics and metabolic disease status are presented in **Table 3.1**. Forty-two participants with biopsy or ultrasound proven NAFLD completed baseline measurements; 40% males and 60% females, of whom 43% had diagnosed T2DM and 55% met the NCEP ATP III[402] criteria for the MetS. The mean age of participants was 52.3 ± 12.6 years, and female participants were significantly older than male participants (56.5 ± 9.5 years vs. 46.2 ± 14.4 years, p=0.008). The majority of participants were recruited from the Alfred Hospital, followed by the Royal Melbourne Hospital and Eastern Health (17, 14 and 11 participants, respectively). Of the participants included in this study, 45.2% were of Asian ethnicity, 31% were European, 19% were Australian and almost 5% were Middle-Eastern. Exactly two-thirds (28/42, 66.7%) of participants were either married, in a defacto relationship or living with a partner, while the remaining one-third (14/42, 33.3%) were single or divorced. A total of 81% of participants reported that they lived with their partner or with family, while 14% lived alone and 5% lived with friends. Employment status varied throughout the group; 38.1% were employed full-time, 14.3% employed part-time, 9.5% employed as casuals, 16.7% unemployed, and 21.4% retired. Furthermore, above 80% of this NAFLD population reported that their education status consisted of a certificate/diploma, bachelor's degree or post-graduate degree. Of the 18 participants who were diagnosed with T2DM,

one was male and 17 were female. Of these, ten (55%) had the MetS. Of the ten participants with both T2DM and the MetS, one was male and nine were female.

Table 3.1. Demographics of a NAFLD cohort (n=42)

Characteristics	
Sex n (M/F)	17 / 25
Diabetes n (Y/N)	18 / 24
Metabolic Syndrome n (Y/N)	23 / 19
Smoking status n (%)	
Current Smoker	0 (0)
Past Smoker	14 (33.3)
Never smoked	28 (66.7)
Recruitment Site n (%)	
Alfred Health	17 (41)
Eastern Health	11 (26)
RMH	14 (33)
Ethnicity n (%)	
Australian	8 (19)
North-West European	6 (14.3)
Southern or Eastern European	7 (16.7)
South-East Asian	10 (23.8)
Chinese Asian	6 (14.3)
Southern or Central Asian	3 (7.1)
Middle Eastern	2 (4.8)
Marital Status n (%)	
Single	9 (21.4)
Married	23 (54.8)
Defacto	4 (9.5)
Living with partner	1 (2.4)
Divorced	5 (11.9)
Living Arrangement n (%)	
Living with spouse	17 (40.5)
Living with family	17 (40.5)
Living with friends	2 (4.8)
Living alone	6 (14.3)
Work Status n (%)	
Employed full-time	16 (38.1)
Employed part-time	6 (14.3)
Employed casual	4 (9.5)
Unemployed	7 (16.7)
Retired	9 (21.4)
Level of Education n (%)	
Didn't complete secondary School	3 (7.1)
Completed secondary school	3 (7.1)
Apprentice/Trade	2 (4.8)
Certificate/Diploma	10 (23.8)
Bachelor's Degree	16 (38.1)
Post-graduate Degree (Masters)	8 (19)

3.4.2 Anthropometry and body composition

Baseline anthropometry and body composition measurements of the NAFLD cohort, based on sex and the presence of the MetS are presented in **Table 3.2.** For the whole cohort, mean weight was 88.8 ± 22.7kg and BMI was 32.2 ± 6.2kg/m^2. Of the total sample, 7.1% were classified as healthy weight (BMI 18-25kg/m^2), 33.3% were classified as overweight (BMI 25-30kg/m^2) and 59.5% were classified as obese (BMI >30kg/m^2), as defined by the World Health Organisation.[400] Bioelectrical impedance analysis (BIA) was used to measure mean fat mass (%) and fat mass index (FMI), which were above mean cut-off points according to NHANES III estimates for body composition[401] (40.1 ± 7.8% and 12.2 ± 6.5kg/m^2, respectively), as was visceral fat (VF) (3.0 ± 2.2L).

There were some significant differences noted between males and females for anthropometry and body composition also presented in **Table 3.2**. Briefly, females had significantly higher percentage FM and FMI than their male counterparts. As expected, males were significantly taller and heavier (body weight), with higher WHR, NC and VF than females. Males also had a higher FFM, FFMI and skeletal muscle mass than female participants at baseline.

There were no significant differences in anthropometry or body composition measurements between individuals with or without the MetS at baseline.

Table 3.2. Baseline anthropometry and body composition characteristics of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

Characteristics	RR	Total		Male		Female		p^g	MetS		Non-MetS		p^m
		n		n		n			n		n		
Age (y)[a]		42	52.36 (12.65)	17	46.24 (14.43)	25	56.52 (9.48)	0.008*	23	52.04 (14.26)	19	52.82 (10.21)	0.847
Anthropometry													
Height (cm)[a]	-	42	165 (10.0)	17	172 (10.0)	25	161 (7.00)	0.001*	23	165 (11.0)	19	166 (8.00)	0.828
Weight (kg)[a]	-	42	88.86 (22.74)	17	97.24 (19.98)	25	83.16 (23.10)	0.048*	23	88.18 (20.72)	19	89.86 (26.07)	0.818
BMI (kg/m2)[a]	18.5-25.0 kg/m^2	42	32.22 (6.27)	17	32.57 (4.98)	25	31.98 (7.10)	0.768	23	32.01 (4.79)	19	32.53 (8.12)	0.796
WC (cm)[b]	M <102 F<88	42	103.48 (21.30)	17	106.40 (21.25)	25	100.40 (22.35)	0.091	23	105.00 (20.65)	19	100.70 (25.80)	0.990
HC (cm)[b]	-	42	107.55 (15.15)	17	106.30 (15.90)	25	108.50 (19.00)	0.778	23	108.50 (14.25)	19	106.60 (24.95)	0.980
WHR[a]	M>0.9 F>0.85	42	0.97 (0.06)	17	1.02 (0.04)	25	0.94 (0.05)	0.001*	23	0.98 (0.06)	19	0.96 (0.05)	0.548
NC (cm)[a]	-	42	39.27 (4.94)	17	42.94 (3.79)	25	36.77 (4.01)	0.001*	23	40.31 (4.22)	19	37.74 (5.63)	0.097
Blood pressure[a]													
Systolic	<120	42	126.52 (16.26)	17	123.41 (9.80)	25	128.64 (19.39)	0.258	23	129.32 (18.48)	19	122.41 (11.60)	0.180
Diastolic	<80	42	83.12 (8.60)	17	82.53 (6.11)	25	83.52 (10.05)	0.719	23	82.16 (10.18)	19	84.53 (5.54)	0.337
Heart Rate[a]	60-100	40	73.69 (12.77)	16	69.91 (11.73)	24	76.21 (13.05)	0.128	23	72.06 (11.46)	17	76.40 (14.71)	0.304
Body Composition													
Fat mass (kg)[b]		42	31.40 (15.22)	17	30.00 (17.94)	25	34.30 (15.90)	0.384	23	34.50 (15.01)	19	31.19 (20.76)	0.691
Fat mass (%)[a]		42	40.07 (7.83)	17	33.59 (5.66)	25	44.48 (5.79)	0.001*	23	38.98 (7.30)	19	41.68 (8.52)	0.277
Fat free mass (kg)[b]		42	50.22 (21.96)	17	63.70 (11.27)	25	42.70 (9.43)	0.001*	23	52.43 (21.87)	19	45.00 (22.05)	0.929
Fat mass index (kg/m2)[b]		42	12.20 (6.45)	17	10.00 (5.10)	25	12.60 (5.80)	0.019*	23	12.30 (5.60)	19	12.00 (8.00)	0.778
Fat free mass index (kg/m2)[a]		42	19.09 (2.81)	17	21.45 (1.95)	25	17.49 (2.08)	0.001*	23	19.43 (2.59)	19	18.59 (3.11)	0.348
Skeletal muscle mass (kg)[b]		42	23.90 (12.98)	17	31.30 (6.15)	25	19.50 (4.95)	0.001*	23	25.70 (12.40)	19	20.50 (14.30)	0.778
Visceral fat (L)[b]		35	3.00 (2.20)	15	4.30 (2.70)	20	2.95 (1.22)	0.019*	19	3.00 (2.30)	16	3.05 (1.72)	0.678

RR, Reference Range; MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference. [a]parametric data presented as mean (SD) [b]non-parametric data presented as median (IQR). [g]p-values for comparing differences between males and females at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data

3.4.3 Biochemical profile

The biochemistry profile of the NAFLD cohort, stratified by sex and the presence of the MetS are presented in **Table 3.3**. Serum ALT levels were elevated above normal levels (>40U/L) in all groups. This was as expected considering that elevated ALT (>30U/L) was part of the inclusion criteria for the trial. Serum AST was within normal limits across all groups except for females wherein it was raised above normal levels (36U/L). Conversely, GGT was elevated above the reference range for all groups except females (who were within normal limits). ALP and bilirubin levels were also within their reference ranges across all groups. There were no significant differences in liver enzymes between males and females, or MetS and non-MetS groups at baseline.

In this NAFLD cohort, females had higher levels of total cholesterol, HDL, and transferrin levels than males (1.3 (0.3) mmol/L vs. 1.0 (0.2) mmol/L, p =0.001, and 2.9 ± 0.4g/L vs. 2.4 ± 0.4g/L, p =0.001, respectively). In contrast, males had higher levels of transferrin saturation and ferritin than females (32.5 ± 12.5% vs. 23.1 ± 9.4%, p =0.009, and 253.0 (297.5) ug/L vs. 108.0 (134.5)ug/L, p =0.006, respectively).

Participants with the MetS had significantly higher fasting insulin (16.8 (14.2) mIU/L vs. 12.7 (5.9) mIU/L, p =0.026), HOMA-IR (4.9 (5.1) vs. 3.0 (1.6), p =0.02) and triglyceride levels (1.8 (0.9) vs. 1.2 (0.5), p =0.012), and lower HDL cholesterol (1.0 (0.3) mmol/L vs. 1.3 (0.3) mmol/L, p =0.0005) than those without the MetS. Participants with the MetS also had higher C-peptide levels than non-MetS NAFLD participants ($p<0.05$), in line with elevated insulin and IR, as well as MetS diagnostic criteria.

Table 3.3. Baseline biochemistry characteristics of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

Biomarkers	Reference Ranges	Total (n=42)	Male (n=17)	Female (n=25)	P[g]	MetS (n=23)	Non-MetS (n=19)	P[m]
ALT[b]	(0-40 U/L)	48.50 (43.00)	47.00 (61.00)	50.00 (38.50)	0.80	48.00 (46.50)	55.00 (39.00)	0.828
AST[b]	(0-35 U/L)	32.00 (25.50)	27.00 (28.50)	36.00 (23.50)	0.40	33.00 (28.00)	31.00 (25.50)	0.969
GGT[b]	(0-62 U/L)	67.00 (105.50)	75.00 (85.50)	58.00 (115.00)	0.71	68.00 (98.50)	66.00 (119.00)	0.818
ALP[b]	(30-110 U/L)	86.50 (30.00)	85.00 (20.00)	92.00 (39.50)	0.25	85.00 (26.50)	94.00 (34.00)	0.599
Bilirubin[b]	(2-21 µmol/L)	12.00 (7.25)	12.00 (11.50)	12.00 (7.00)	0.488	10.00 (6.00)	13.00 (9.50)	0.083
Total Protein[a]	(60-80 g/L)	76.40 (5.64)	75.12 (4.57)	39.20 (3.80)	0.227	76.88 (5.78)	75.71 (5.52)	0.514
Albumin[a]	(33-46 g/L)	39.69 (3.29)	40.41 (2.26)	39.20 (3.79)	0.204	40.40 (3.24)	38.65 (3.16)	0.09
Globulin[b]	(24-39 g/L)	36.00 (5.50)	36.00 (6.00)	37.00 (7.50)	0.114	37.00 (6.00)	36.00 (6.00)	0.738
Glucose[b]	(3.5-6.0 mmol/L)	5.80 (1.70)	5.30 (1.25)	6.40 (2.10)	0.106	6.00 (1.90)	5.40 (1.75)	0.293
Insulin[b]	(2-20 mIU/L)	14.75 (8.28)	15.00 (7.70)	14.40 (11.50)	0.918	16.80 (14.20)	12.70 (5.95)	0.026*
HOMA-IR[b]		3.80 (2.92)	3.53 (2.29)	4.26 (4.24)	0.391	4.90 (5.13)	3.00 (1.62)	0.020*
HbA1c (%)[b]	(> 6.0)	5.90 (1.10)	5.80 (0.90)	6.00 (1.20)	0.218	5.90 (1.15)	5.90 (1.25)	0.238
Cholesterol[b]	(0-5.5 mmol/L)	4.85 (2.33)	4.50 (1.40)	4.90 (2.25)	0.599	4.50 (2.10)	4.90 (2.40)	0.969
HDL[b]	(> 1.0 mmol/L)	1.15 (0.40)	1.00 (0.20)	1.30 (0.30)	0.00*	1.00 (0.25)	1.30 (0.25)	<0.001*
LDL[a]	(< 3.5 mmol/L)	3.03 (1.30)	3.02 (1.05)	3.03 (1.47)	0.995	3.11 (1.45)	2.90 (1.09)	0.606
Triglycerides[b]	(< 2.0 mmol/L)	1.60 (0.97)	1.60 (0.85)	1.60 (1.07)	0.867	1.80 (0.95)	1.20 (0.50)	0.012*
Iron[a]	(7-32 µmol/L)	17.21 (5.67)	18.76 (5.82)	16.16 (5.43)	0.146	17.28 (5.76)	17.12 (5.71)	0.929
Transferrin[a]	(1.81-3.31 g/L)	2.71 (0.47)	2.41 (0.36)	2.91 (0.42)	0.00*	2.70 (0.53)	2.73 (0.37)	0.851
Transferrin Sat[a]	(< 50.0%)	26.83 (11.59)	32.35 (12.51)	23.08 (9.43)	0.009*	27.08 (11.70)	26.47 (11.79)	0.870
Ferretin[b] (30-600 ug/L)		147.00 (216.50)	253.00 (297.50)	108.00 (134.50)	0.006*	153.00 (242.00)	110.00 (222.50)	0.481
C-peptide[b] (mmol/L)		1058.50 (455.00)	1030.00 (503.50)	1066.00 (480.00)	0.608	1135.00 (463.50)	919.00 (464.50)	0.022*

MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a]parametric data presented as mean (SD) [b]non-parametric data presented as median (IQR). [g]p-values for comparing differences between males and females at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data

3.4.4 Inflammatory Profile

The inflammatory profile of the NAFLD cohort and stratified for sex and the presence of the MetS are presented in **Table 3.4**. Compared to males, female participants had higher serum levels of inflammatory markers; hs-CRP, IL-6, resistin and leptin, albeit non-significant, except for leptin (19.8 (18.8) ng/ml vs. 7.3 (8.1) ng/ml, p =0.003, respectively). Adiponectin was also non-significantly higher (more favourable) in females compared to males.

There were no significant differences in the inflammatory profile of the cohort according to their MetS diagnosis. Interestingly, circulating levels of serum TNF-α, IL-6, resistin and leptin were higher in individuals without the MetS than those with the MetS. High sensitivity-CRP was higher in those with the MetS and adiponectin was higher in those without the MetS, albeit not significantly. Unlike other inflammatory markers, circulating adiponectin is usually found in lower levels in individuals with metabolic or chronic disease. This was reflected in the present cohort, in that serum adiponectin was non-significantly higher in individuals without the MetS.

3.4.5 Liver outcomes

Liver outcome measures for this NAFLD cohort and stratified based on sex and the presence of the MetS are presented in **Table 3.5**. This cohort had elevated liver fat (12.2 ± 10%) compared to the range for healthy adults (<5.0%),[11] as defined by ^{1}H-MRS. Males tended to have higher liver fat than females, as did participants with MetS in comparison to those without MetS, though these differences were not significant.

The median LSM score for this NAFLD cohort (6.3kPa) was above the median "normal" LSM result (5.3kPa) indicating that 'moderate/mild fibrosis may be present in this cohort, though cirrhosis is exceptionally unlikely'.[403] There were no significant differences in LSM score across sex or MetS diagnosis.

Table 3.4. Baseline cytokine and adipokine profile of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

Inflammatory marker	Total		Male		Female		P[g]	MetS		Non-MetS		P[m]
	n		n		n			n		n		
hs-CRP[b] (0-3 mg/L)	38	2.35 (4.17)	17	1.90 (4.00)	21	3.60 (4.30)	0.189	23	2.80 (3.95)	15	1.80 (5.15)	0.808
TNF-α[b] (pg/ml)	41	4.40 (2.98)	16	4.53 (2.29	25	3.82 (3.09)	0.534	23	3.87 (2.03)	18	4.80 (3.57)	0.227
IL-6[b] (pg/ml)	41	3.37 (11.81)	16	2.73 (10.38)	25	3.67 (15.90)	0.055	23	3.25 (12.53)	18	3.86 (11.88)	0.486
Adiponectin[b] (µg/mL)	41	11.33 (13.87)	16	8.89 (5.52)	25	13.15 (14.42)	0.052	23	10.33 (11.87)	18	12.26 (16.04)	0.344
Resistin[b] (ng/mL)	41	36.28 (15.92)	16	33.01 (22.06)	25	37.72 (17.31)	0.926	23	36.28 (18.48)	18	38.05 (27.20)	0.572
Leptin[b] (ng/ml)	41	12.47 (16.32)	16	7.28 (8.09)	25	19.83 (18.83)	0.003*	23	10.29 (13.78)	18	18.10 (16.96)	0.093

MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome; hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6. Values >10 mg/L hs-CRP excluded from analysis (n=3). [a]parametric data presented as mean (SD) [b]non-parametric data presented as median (IQR). [g]p-values for comparing differences between males and females at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data.

Table 3.5. Baseline liver fat and stiffness measurements of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

	RR	Total		Male		Female		P[g]	MetS		Non-MetS		P[m]
		n		n		n			n		n		
Liver Fat (%)[a]	< 5.0%	33	11.39 (9.51)	13	11.37 (12.35)	20	11.52 (7.49)	0.919	21	12.04 (9.98)	12	10.22 (8.93)	0.604
LSM (kPa)[b]	< 5.3 kPa	42	6.30 (6.80)	17	5.30 (4.45)	25	7.20 (6.60)	0.065	25	6.50 (7.10)	17	6.30 (5.85)	0.749

MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome; LSM, Liver Stiffness Measure. [a]parametric data presented as mean (SD). [b]non-parametric data presented as median (IQR). [g]p-values for comparing differences between males and females at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data.

3.4.6 Dietary intake

Baseline dietary intakes of the NAFLD cohort, described by sex and the presence of the MetS, are presented in **Table 3.6.** Median energy intake for the cohort was 7682.9 (3965.1) kJ and with higher daily intakes in males compared to females (8862.19 (5259.22) kJ vs. 7107.28 (3437.45) kJ, p =0.005). Total fat as a percentage contribution to total energy was 35.23 (4.95) %, mean protein as a percentage of total energy was 18.95 (3.89) % and mean carbohydrate as a percentage of total energy was 42.27 (6.94) %. In terms of protein, carbohydrate and fat intake as a percentage contribution to total energy, there were no significant differences between males and females. Interestingly, this cohort was not meeting the energy intake requirements for their mean age group, although this may be attributable to under-reporting of dietary intake in the 3-day food diaries provided by participants. Participants were however, meeting the upper limit for total fat intake (20-35%E) and protein intake (15-25%E), as defined by NHMRC.[404] Participants were consuming mostly monounsaturated fatty acids (MUFAs), followed by saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) as a percentage of total energy intake (14.20 (4.23)%, 12.01 (3.29) %, 5.41 (3.12) %, respectively). Consumption of SFAs were above the recommended range set by NHMRC guidelines to reduce chronic disease risk (<10%E) and PUFA intake was within range of the recommended amount (6-11%E).[404] This populations intake of carbohydrate as a percentage of total energy was below the carbohydrate recommendations (45-65%E) for reduction of chronic disease risk.[404]

On average, participants were consuming 24.25 (10.40) g fibre per day, almost meeting the recommended daily intake for fibre (25-30g per day). As a group, total sugar intake as a percentage of total energy was 16.83 (9.14) %, higher than the recommended intake (<10%E). Total cholesterol intake for this cohort was 271.77 (202.12) mg, which meets the upper limit of the RDI for cholesterol in a healthy population (300mg per day), though above the recommended intake for individuals at risk of heart disease (200-250mg per day).

Within this cohort, all participants reported to be consuming adequate amounts of very long chain omega-3 fatty acids (0.14 (0.19) g per day), with males consuming as high as 0.26 (0.54) g and females consuming slightly less 0.13 (0.15) g. This finding was surprising in an overweight cohort, considering the adequate intake (AI) for very long chain omega-3 fatty acids is 0.145 g per day. This same trend was observed for alpha linoleic acid (ALA), with the AI of 1.2g per day met for all participants (1.31 (1.46) g), greater in males (1.51 (1.18) g) and compared to females (1.17 (1.72) g). The AI for linoleic acid (12 g per day) was not met in this population (9.43 (8.62) g). The AIs for ALA and linoleic acid were based on the Nutrient Reference Values for Australia and New Zealand, taken from an analysis of the National Nutrition Survey of Australia of 1995.[405, 406]

Vitamin C intake was well above the recommended intake of 45mg per day in the total cohort (81.37 (68.91) mg) and across all groups. Vitamin E intake was also above the recommended intake of

10mg per day for the total cohort (11.22 (7.94) mg) and across all groups. This finding was reflected in levels of tocopherol alpha, though to a lesser extent. RDI's of sodium and iron were met within the total cohort and across all groups, and as expected male participants were consuming a significantly higher amount of each micronutrient in comparison to female participants (2829.35 (1015.46) mg vs. 2124.27 (844.48) mg, p =0.019, and 12.68 (5.25) mg vs. 8.69 (4.94) mg, p =0.009, respectively). Daily intake of potassium, magnesium, calcium and zinc were below corresponding RDI's. As expected, males also consumed higher amounts of these micronutrients than females at baseline; potassium (3424.23 (1020.44) mg vs. 2618.37 (1097.41) mg, p =0.021), magnesium (403.06 (162.86) mg vs. 300.87 (118.60) mg, p =0.023), calcium (1000.88 (581.51) mg vs. 677.91 (277.36) mg, p =0.022), and zinc (11.57 (6.86) mg vs. 7.74 (4.39) mg, p =0.004).

There were no significant differences in the composition of the diet of individuals with or without the MetS and NAFLD.

Table 3.6. Baseline dietary intake of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

	Total (n=42)	Male (n=17)	Female (n=25)	P[g]	MetS (n=23)	Non-MetS (n=19)	P[m]
Macronutrients							
Energy (kJ)[b]	7682.94 (3965.08)	8862.19 (5259.22)	7107.28 (3437.45)	0.005*	7449.70 (4444.17)	8606.82 (3613.77)	0.672
Protein (g)[a]	94.28 (34.84)	116.46 (37.68)	79.19 (23.26)	0.0005*	96.73 (39.79)	90.67 (26.68)	0.586
Protein (% of total E)	18.95 (3.89)	19.86 (4.39)	18.34 (3.48)	0.219	19.38 (4.13)	18.33 (3.54)	0.396
Carbohydrate (g)[a]	219.22 (81.29)	252.16 (87.35)	196.81 (70.06)	0.028*	215.99 (89.40)	223.96 (70.02)	0.760
Carbohydrate (% of total E)	42.27 (6.94)	40.96 (8.64)	43.16 (5.53)	0.320	41.62 (8.11)	43.23 (4.82)	0.467
Sugars (g)[b]	77.35 (60.20)	78.83 (66.92)	74.98 (56.23)	0.254	63.55 (74.59)	82.21 (37.62)	0.405
Sugars (% of total E)	16.83 (9.14)	14.54 (7.58)	17.93 (9.35)	0.187	15.25 (8.40)	19.74 (8.72)	0.276
Added sugars (% of total E)	5.22 (5.60)	5.43 (6.04)	5.01 (6.93)	0.556	5.43 (4.90)	4.74 (7.00)	0.810
Total fat (g)[b]	72.79 (41.58)	94.62 (73.12)	68.20 (38.50)	0.042*	68.56 (39.94)	73.61 (42.83)	0.749
Total fat (% of total E)	35.23 (4.95)	35.41 (8.11)	35.03 (4.02)	0.450	34.76 (6.04)	35.29 (5.06)	0.848
Saturated Fat (g)[a]	28.54 (14.39)	34.79 (17.61)	24.28 (10.02)	0.018*	30.19 (16.75)	26.11 (9.98)	0.373
Saturated fat (% of total fat)	37.87 (8.19)	38.04 (6.92)	37.76 (9.09)	0.916	39.56 (8.43)	35.40 (7.38)	0.107
Saturated fat (% of total E)	12.01 (3.29)	12.42 (3.89)	11.73 (2.86)	0.509	12.60 (3.42)	11.14 (2.97)	0.160
Mono-unsaturated Fat (g)[b]	27.14 (18.05)	35.36 (30.61)	24.80 (16.06)	0.023*	25.53 (18.50)	31.63 (20.17)	0.654
Mono-unsaturated fat (% of total fat)	44.00 (5.71)	44.23 (6.20)	43.89 (6.55)	0.530	42.74 (4.17)	46.03 (6.32)	0.056
Mono-unsaturated fat (% of total E)	14.20 (4.23)	14.48 (4.99)	14.07 (4.36)	0.599	13.11 (4.22)	15.10 (3.86)	0.170
Poly-unsaturated Fat (g)[b]	11.31 (10.26)	12.77 (10.56)	10.15 (10.50)	0.170	10.72 (11.39)	12.67 (10.35)	0.513
Poly-unsaturated Fat (% of total fat)	17.10 (9.25)	16.44 (9.58)	18.36 (8.88)	0.405	15.21 (7.90)	19.76 (8.61)	0.75
Poly-unsaturated Fat (% of total E)	5.41 (3.12)	4.71 (2.05)	5.49 (3.32)	0.530	4.61 (2.37)	6.30 (2.95)	0.067
Dietary Fibre (g)[a]	24.25 (10.40)	26.78 (10.71)	22.54 (10.05)	0.199	23.06 (10.87)	26.01 (9.72)	0.374
Cholesterol (mg)[b]	271.77 (202.12)	290.33 (246.68)	254.60 (210.69)	0.098	285.87 (226.65)	269.23 (188.11)	0.635
Alcohol (g)[b]	0.00 (0.00)	0.00 (0.10)	0.00 (0.00)	0.418	0.00 (0.00)	0.00 (0.10)	0.396
Tocopherol Alpha (mg)[b]	9.70 (6.39)	11.17 (11.53)	9.39 (5.89)	0.141	9.34 (5.38)	10.08 (8.55)	0.513
Linoleic Acid (g)[b]	9.43 (8.62)	10.78 (9.34)	8.19 (8.64)	0.127	9.01 (9.78)	10.76 (8.44)	0.530
ALA (g)[b]	1.31 (1.46)	1.51 (1.18)	1.17 (1.72)	0.949	1.17 (0.87)	1.45 (1.98)	0.729

EPA (g)[b]	0.03 (0.06)	0.07 (0.11)	0.03 (0.03)	0.287	0.05 (0.06)	0.02 (0.03)	0.115
DPA (g)[b]	0.05 (0.06)	0.06 (0.11)	0.04 (0.06)	0.204	0.06 (0.06)	0.04 (0.06)	0.282
DHA (g)[b]	0.05 (0.10)	0.07 (0.34)	0.05 (0.10)	0.233	0.07 (0.12)	0.05 (0.09)	0.391
Very Long Chain Omega-3 Fatty Acids (g)[b]	0.14 (0.19)	0.26 (0.54)	0.13 (0.15)	0.155	0.22 (0.23)	0.12 (0.13)	0.144
Trans Fatty Acids (g)[a]	1.26 (0.73)	1.50 (0.89)	1.10 (0.57)	0.083	1.34 (0.79)	1.16 (0.64)	0.450
Micronutrients							
Vitamin C (mg)[b]	81.37 (68.91)	88.18 (68.85)	79.47 (68.91)	0.691	79.47 (59.60)	88.18 (182.41)	0.324
Vitamin E (mg)[b]	11.22 (7.94)	12.23 (13.20)	10.66 (6.66)	0.155	10.66 (5.71)	11.49 (9.83)	0.654
Sodium (mg)[a]	2409.66 (970.86)	2829.35 (1015.46)	2124.27 (844.48)	0.019*	2304.67 (1017.57)	2564.06 (905.32)	0.402
Potassium (mg)[a]	2944.55 (1127.65)	3424.23 (1020.44)	2618.37 (1097.41)	0.021*	2928.88 (1058.91)	2967.59 (1255.23)	0.915
Magnesium (mg)[a]	342.23 (145.47)	403.06 (162.86)	300.87 (118.60)	0.023*	336.84 (152.46)	350.16 (138.73)	0.775
Calcium (mg)[b]	770.11 (445.12)	1000.88 (581.51)	677.91 (277.36)	0.022*	756.24 (546.75)	782.49 (427.73)	0.908
Iron (mg)[b]	9.93 (5.16)	12.68 (5.25)	8.69 (4.94)	0.009*	10.53 (5.26)	9.29 (5.51)	0.420
Zinc (mg)[a]	9.23 (5.42)	11.57 (6.86)	7.74 (4.39)	0.004*	11.26 (5.17)	9.85 (3.90)	0.345

MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome; ALA, Alpha Linolenic Acid; EPA, Eicosapentaenoic Acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid. [a]parametric data presented as mean (SD) [b]non-parametric data presented as median (IQR). [g]p-values for comparing differences between males and females at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data.

3.4.7 Food Group Intake

The food group intake of this NAFLD cohort is presented in **Table 3.7**, characterised by sex and presence of the MetS. The total cohort of participants were not meeting the recommended serves for all food groups, with the exception of meat and meat alternatives for which males were consuming above the recommended dietary intake (RDI) and females were consuming adequate serves per day.

The entire cohort did not meet the recommended intake for grains; particularly wholegrain intake for both males and females were low (male RDI 6.0 serves/day, actual intake 1.0 (2.2) serves/day and female RDI 4.0 serves/day, actual intake 0.9 (1.8) serves/day). The consumption of refined grains was remarkably high for this cohort (5.1 (2.7) serves/day), with males consuming more refined grains than females, albeit not significantly. Participants were consuming approximately 1.5 serves less than the RDI of vegetables per day and 1 serve below the RDI for fruit per day. Interestingly, males consumed twice the amount of fruit per day compared to females (NS). At baseline, males were also consuming significantly higher servings of grains per day than females (7.4 (4.4) vs. 5.6 (1.9), p =0.023, respectively).

Recommended intakes for dairy were not met within this cohort, males consumed approximately 1 serve less than required and females approximately 2.5 serves less than required per day. Dairy consumption in both males and females consisted mostly of milk and cheese with males consuming more of both foods, albeit not significantly. Participants were consuming 6.9 (6.3) serves (or teaspoons) of oil equivalents (including unsaturated spreads, oils and unhydrogenated vegetable oils) per day which is approximately 32g. On the other hand, participants were also consuming considerable amounts of solid fat equivalents, mostly made up of butter (and fully or partially hydrogenated oils, shortening, palm oil and coconut oil) with 8.1 serves (or teaspoons) equal to approximately 39g.

There were no significant differences in the food group intake of individuals with or without the MetS at baseline.

Table 3.7. Baseline food group intake of the NAFLD cohort, split by sex or presence of metabolic syndrome (MetS)

Food Group	ADG[272] (Serves per day) Male	Female	Total (n=42)	Male (n=17)	Female (n=25)	P[g]	MetS (n=23)	Non-MetS (n=19)	P[m]
Grains[b]			6.60 (3.23)	7.37 (4.37)	5.55 (1.89)	0.023*	6.64 (4.12)	5.97 (2.60)	0.888
Wholegrains[b]	6.0	4.0	0.97 (1.83)	1.00 (2.20)	0.93 (1.76)	0.626	1.53 (1.90)	0.61 (1.71)	0.227
Refined Grains[b]			5.13 (2.65)	6.07 (4.26)	5.03 (1.89)	0.093	5.06 (2.72)	5.53 (3.17)	0.654
Vegetables[a]	5.5	5.0	3.78 (2.61)	3.99 (2.50)	3.63 (2.72)	0.665	3.51 (2.46)	4.18 (2.84)	0.418
Fruit[b]	2.0	2.0	0.92 (1.77)	1.21 (1.79)	0.61 (1.35)	0.228	0.89 (1.50)	1.21 (2.22)	0.530
Meat and meat alternatives[b]	2.5	2.0	2.18 (1.81)	3.42 (3.99)	1.96 (1.33)	0.013*	2.25 (1.78)	1.97 (2.33)	0.908
Red meats[b]			0.51 (1.24)	0.55 (1.65)	0.47 (1.15)	0.524	0.55 (1.35)	0.45 (1.08)	0.413
Processed meats[b]			0.00 (0.25)	0.00 (0.44)	0.00 (0.23)	0.780	0.00 (0.21)	0.02 (0.30)	0.207
Poultry[b]			0.43 (0.65)	0.55 (0.61)	0.40 (0.73)	0.193	0.47 (0.80)	0.43 (0.48)	0.479
Eggs[a]			0.23 (0.20)	0.20 (0.19)	0.25 (0.21)	0.417	0.21 (0.20)	0.26 (0.20)	0.408
Seafood									
High long chain omega-3[b]			0.00 (0.03)	0.00 (0.20)	0.00 (0.01)	0.324	0.00 (0.09)	0.00 (0.01)	0.295
Low long chain omega-3[b]			0.00 (0.40)	0.00 (0.47)	0.00 (0.39)	0.725	0.00 (0.47)	0.00 (0.20)	0.328
Legumes[b]			0.00 (0.03)	0.00 (0.06)	0.00 (0.09)		0.00 (0.00)	0.00 (0.22)	
Nuts[b]			0.18 (0.75)	0.35 (1.94)	0.15 (0.38)	0.466	0.00 (0.96)	0.28 (0.60)	0.320
Dairy[b]	2.5	4.0	1.38 (1.09)	1.77 (1.70)	1.28 (0.71)	0.205	1.45 (1.16)	1.23 (1.12)	0.635
Milk[b]			0.89 (1.07)	1.00 (1.70)	0.65 (0.85)	0.200	0.97 (1.35)	0.65 (0.73)	0.412
Yoghurt[b]			0.00 (0.16)	0.00 (0.02)	0.00 (0.02)	0.542	0.00 (0.00)	0.00 (0.22)	0.146
Cheese[b]			0.36 (0.75)	0.46 (0.99)	0.22 (0.61)	0.422	0.36 (0.73)	0.36 (0.77)	0.876
Oil Equivalents[b]			6.92 (6.25)	7.79 (8.04)	6.07 (4.89)	0.121	6.51 (6.53)	8.08 (6.43)	0.434
Solid fat equivalents[b]			8.13 (4.61)	8.24 (9.63)	7.49 (4.71)	0.276	6.82 (6.51)	8.70 (3.13)	0.828
Added Sugars[b]			6.40 (7.55)	7.40 (11.14)	6.05 (7.75)	0.720	6.76 (8.06)	6.05 (7.91)	0.729
Alcoholic beverages[b]	<2.0	<2.0	0.00 (0.00)	0.00 (0.04)	0.00 (0.00)	0.418	0.00 (0.00)	0.00 (0.04)	0.396

ADG, Australian Dietary Guidelines; MetS, Metabolic Syndrome; Non-MetS, Non-Metabolic Syndrome. [a]parametric data presented as mean (SD). [b]non-parametric data presented as median (IQR). Diet composition data were exported from FoodWorks 9™ for analysis. Nutrient and food group definitions, classifications, serving sizes were based on the settings in Xyris, Foodworks 9™ software and can be found in **Appendix 7**. [g]p-values for comparing differences between males and females at baseline;

independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. [m]p-values for comparing differences between participants diagnosed with or without the metabolic syndrome at baseline; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data.

3.4.8 Associations of Anthropometric Measurements and Biochemical Markers with Inflammatory cytokines and adipokines in a NAFLD cohort

Pearson and spearman rho correlations were performed to assess the relationship between anthropometric and biochemical variables with cytokine and adipokine markers in this NAFLD cohort. Data presented in **Table 3.8** reflects the strength, direction and significance of the associations between variables. Additional, non-significant variables are presented in **Appendix 11; Supplementary Table 1**.

At baseline, BMI, hip circumference, fat mass (kg and %), fat mass index, total protein and globulin each had a significant ($p < 0.05$) moderate and positive correlation with hs-CRP ($r = 0.40, 0.38, 0.36, 0.44, 0.46, 0.35$ and 0.49, respectively). Weight, waist and hip circumference, waist-to-hip ratio, fat-free mass, fat free mass index, skeletal muscle mass and glucose each had a significant ($p < 0.05$) moderate and positive correlation with TNF-α ($r = 0.35, 0.37, 0.33, 0.35, 0.36, 0.31, 0.34$ and 0.31, respectively). There were no significant correlations between anthropometric and biochemical variables and IL-6 at baseline.

Fat mass (kg and %) and fat mass index each had a significant ($p < 0.01$) strong and positive correlation with adiponectin ($r = 0.51, 0.54$ and 0.51, respectively) and BMI, hip circumference, ALT, AST and liver fat each had a significant ($p < 0.05$) moderate and positive correlation with adiponectin ($r = 0.34, 0.43, 0.33, 0.35$ and 0.46, respectively). Hip circumference, fat mass (kg and %) and fat mass index each had a significant ($P < 0.01$) strong, positive correlation with leptin ($r = 0.58, 0.67, 0.74$ and 0.71, respectively). Conversely, albumin had a significant ($P < 0.01$) moderate and negative correlation with leptin ($r = -0.42$). Weight, BMI, waist circumference, diastolic blood pressure, AST, globulin, insulin, HOMA-IR and HDL each had a significant ($p < 0.05$) moderate and positive correlation with leptin ($r = 0.32, 0.45, 0.41, 0.34, 0.31, 0.40, 0.32, 0.32$ and 0.31, respectively). Weight, BMI, waist circumference, neck circumference and fat mass (kg) each had a significant ($P < 0.05$) moderate, positive correlation with resistin.

Table 3.8. Correlations between anthropometric and biochemical variables at baseline

	Correlation Coefficients					
	hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Weight	0.20	0.35*	-0.10	0.24	0.32*	0.36*
BMI (kg/m²)	0.40*	0.23	-0.12	0.34*	0.45**	0.31*
WC (cm)	0.26	0.37*	-0.08	0.29	0.41**	0.33*
HC (cm)	0.38*	0.33*	-0.02	0.43**	0.58**	0.29
WHR	-0.05	0.35*	-0.03	-0.23	-0.20	0.20
NC (cm)	0.08	0.26	-0.22	-0.01	-0.04	0.32*

Fat Mass (kg)	0.36*	0.27	0.02	0.51**	0.67**	0.36*
Fat Mass (%)	0.44**	0.07	0.17	0.54**	0.74**	0.26
Fat Free Mass (kg)	0.01	0.36*	-0.16	0.02	-0.03	0.28
Fat Mass Index (kg/m^2)	0.46**	0.16	0.07	0.51**	0.71**	0.30
Fat free mass index (kg/m^2)	0.12	0.31*	-0.21	-0.07	-0.06	0.20
Skeletal muscle mass (kg)	0.06	0.34*	-0.19	0.01	-0.02	0.25
Blood Pressure Systolic	-0.23	-0.08	-0.03	0.29	0.01	0.13
Blood Pressure Diastolic	0.07	0.21	0.05	0.17	0.34*	0.27
ALT	0.12	0.06	0.26	0.33*	0.21	0.10
AST	0.23	0.11	0.21	0.35*	0.31*	0.07
Total Protein	0.35*	-0.04	0.13	-0.07	0.15	0.01
Albumin	-0.26	-0.24	-0.25	-0.16	-0.42**	0.02
Globulin	0.49**	0.13	0.21	0.01	0.40**	0.03
Glucose	0.25	0.31*	0.19	0.04	0.21	0.25
Insulin	0.24	0.18	0.10	-0.03	0.32*	0.26
HOMA-IR	0.31	0.22	0.13	0.01	0.32*	0.26
HDL	0.15	-0.09	0.28	0.29	0.31*	-0.06
Liver Fat (%)	0.15	0.06	-0.02	0.46**	0.33	0.05
LSM (kPa)	0.21	-0.07	0.09	-0.11	0.24	0.16

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.
hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

3.4.9 Associations between Inflammatory Markers at Baseline

Spearman rho correlations were performed to determine the association between inflammatory markers, data presented is in **Table 3.9**. Leptin had a significant ($p < 0.05$) moderate and positive correlation with TNF-α and hs-CRP ($r = 0.35$ and 0.47, respectively) and a strong positive correlation with adiponectin ($r = 0.52$).

Table 3.9. Correlations between inflammatory markers at baseline

	TNF-α	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
hs-CRP	0.07	0.27	0.19	0.467**	0.20
TNF-α	-	0.25	0.06	0.355*	0.16
IL-6	-	-	0.15	0.27	0.12
Adiponectin	-	-	-	0.518**	0.16
Leptin	-	-	-	-	0.22

3.4.10 Associations of Nutrients and Food Group Intake with Markers of Inflammation in a NAFLD cohort

Pearson and Spearman rho correlations were performed to determine the association between nutrients and food group intake with cytokine and adipokine markers, and the data presented in **Table 3.10**. Although all of the nutrient and food group variables that were previously presented in this chapter were included in the correlational analysis, the non-significant variables are not presented in the main body of the thesis as they do not contribute to answering the main aims of this study. Instead, they are presented in **Appendix 11; Supplementary Table 2**.

Polyunsaturated fat and linoleic acid intake each had a significant ($p < 0.01$) strong and negative correlation with hs-CRP ($r = -0.55$ and -0.57, respectively). Dietary intake of energy, protein, carbohydrate, total fat, monounsaturated fat, polyunsaturated fat (as % of total energy intake), cholesterol, tocopherol alpha, alpha linoleic acid (ALA), vitamin E, magnesium and zinc each had a significant ($p < 0.05$) moderate and negative correlation with hs-CRP ($r = -0.44$, -0.37, -0.35, -0.38, -0.33, -0.34, -0.33, -0.40, -0.44, -0.43, -0.38 and -0.34, respectively). Saturated fat (as % of total energy intake) had a significant ($p < 0.01$) moderate and positive correlation with IL-6 ($r = 0.42$), whereas polyunsaturated fat (as % of total energy intake) had a significant ($p < 0.05$) moderate and negative correlation with IL-6 ($r = -0.34$). Dietary intake of protein, polyunsaturated fat, tocopherol alpha, linoleic acid, vitamin E, magnesium and calcium each had a significant ($p < 0.05$) moderate and negative correlation with leptin ($r = -0.39$, -0.36, -0.34, -0.39, -0.34, -0.31 and -0.33, respectively). Total fat and saturated fat (both as % of total energy intake), eicosapentaenoic acid (EPA) and very long chain fatty acid intake each had a significant ($p < 0.05$) moderate and positive correlation with TNF-α ($r = 0.34$, 0.31, 0.31 and 0.33, respectively). There were no significant associations between nutrient intake and adiponectin or resistin at baseline.

With regards to the relationship between food group intake and inflammatory markers there were few significant findings observed. Intake of processed meats had a significant ($p < 0.05$) moderate and positive association with TNF-α ($r = 0.36$). Intake of meat and meat alternatives and oil equivalents each had a significant ($p < 0.05$) moderate and negative correlation with hs-CRP ($r = -0.37$ and -0.34, respectively). Intake of refined grains had a significant ($p < 0.05$) moderate and negative correlation with adiponectin ($r = -0.35$).

Food group intake of grains, refined grains and poultry each had a significant ($p < 0.05$) moderate, and negative correlation with leptin ($r = -0.45$, -0.35 and -0.37, respectively). Intake of wholegrains and nuts each had a significant ($p < 0.05$) moderate and negative correlation with resistin ($r = -0.33$ and -0.31, respectively). There were no significant associations between food group intake and IL-6 at baseline.

Table 3.10. Correlations between Nutrient and Food Group Intake with Markers of Inflammation at baseline

	Correlation Coefficients					
	hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Macronutrients						
Energy (kJ)	-0.44**	0.18	0.06	-0.14	-0.28	-0.21
Protein (g)	-0.37*	0.12	-0.02	-0.20	-0.39*	-0.06
Carbohydrate (g)	-0.35*	0.03	0.06	-0.18	-0.26	-0.27
Total fat (g)	-0.38*	0.28	0.20	-0.13	-0.19	-0.16
Total fat (% of total E)	-0.08	0.34*	0.26	-0.11	0.06	0.03
Saturated fat (% of total E)	0.03	0.31*	0.42**	0.12	0.28	0.13
Mono-unsaturated Fat (g)	-0.33*	0.30	0.14	-0.17	-0.18	-0.14
Poly-unsaturated Fat (g)	-0.55**	0.06	-0.05	-0.17	-0.36*	-0.22
Poly-unsaturated Fat (% of total E)	-0.34*	-0.23	-0.34*	-0.12	-0.30	-0.25
Cholesterol (mg)	-0.33*	0.12	0.22	-0.13	-0.10	0.15
Tocopherol Alpha (mg)	-0.40*	0.09	0.12	-0.10	-0.34*	-0.22
Linoleic Acid (g)	-0.57**	0.06	-0.04	-0.20	-0.39**	-0.23
ALA (g)	-0.44**	0.01	-0.09	-0.12	-0.18	-0.13
EPA (g)	-0.06	0.31*	0.16	0.05	0.09	-0.23
Very Long Chain Omega-3 Fatty Acids; DHA:EPA:DPA (g)	-0.16	0.33*	0.14	-0.05	0.05	-0.21
Vitamin E (mg)	-0.43**	0.07	0.10	-0.11	-.034*	-0.25
Magnesium (mg)	-0.38*	-0.06	0.04	-0.11	-0.31*	-0.17
Calcium (mg)	-0.17	-0.15	0.21	0.06	-0.33*	-0.06
Zinc (mg)	-0.34*	0.10	0.08	-0.12	-0.30	-0.06
Grains	-0.31	0.02	-0.06	-0.28	-0.45**	-0.33*
Refined Grains	-0.16	0.12	0.04	-0.35*	-0.35*	-0.30
Meat and meat alternatives	-0.37*	0.20	-0.07	-0.17	-0.27	0.07
Processed meats	-0.08	0.36*	-0.10	0.19	0.18	0.22
Poultry	-0.17	-0.07	-0.13	-0.13	-0.37*	0.18
Nuts	-0.28	-0.10	-0.08	-0.20	-0.29	-0.31*
Oil Equivalents	-0.34*	0.12	-0.01	-0.12	-0.30	-0.23
****. Correlation is significant at the 0.01 level (2-tailed).**						
***. Correlation is significant at the 0.05 level (2-tailed).**						

R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.
ALA, Alpha Linolenic Acid; EPA, Eicosapentaenoic Acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid.

3.4.11 Summary of Significant (Positive and Negative) Correlations

There are many outcomes presented in this chapter investigating the relationship between inflammatory markers hs-CRP, TNF-α, IL-6, adiponectin, leptin, resistin and the various anthropometric, clinical, and dietary factors. Thus, the table below provides an overall summary table displaying the significant relationships as a '+' or a '-' sign. The key for each symbol is presented in the footnotes of the table.

Variable	**Correlation Coefficients**					
	hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Anthropometry, Haemodynamic Measures and Body Composition						
Weight		+			+	+
BMI (kg/m^2)	+			+	+ +	+
WC (cm)		+			+ +	+
HC (cm)	+	+		+ +	+ +	
WHR		+				
NC (cm)						+
Fat Mass (kg)	+			+ +	+ +	+
Fat Mass (%)	+ +			+ +	+ +	
Fat Free Mass (kg)		+				
Fat Mass Index (kg/m^2)	+ +			+ +	+ +	
Fat free mass index (kg/m^2)		+				
Skeletal muscle mass (kg)		+				
Blood Pressure Diastolic					+	
Biochemistry and Inflammatory Markers						
ALT				+		
AST				+	+	
Total Protein	+					
Albumin					- -	
Globulin	+ +				+ +	
Glucose		+				
Insulin					+	
HOMA-IR					+	
HDL					+	
Liver Fat (%)				+ +		
hs-CRP					+ +	
TNF-α					+	
Adiponectin					+ +	
Nutrients and Food Groups						
Energy (kJ)	-					

Protein (g)	-				-	
Carbohydrate (g)	-					
Total fat (g)	-					
Total fat (% of total E)		+				
Saturated fat (% of total E)		+	+ +			
Mono-unsaturated Fat (g)	-					
Poly-unsaturated Fat (g)	- -				-	
Poly-unsaturated Fat (% of total E)	-		-			
Cholesterol (mg)	-					
Tocopherol Alpha (mg)	-				-	
Linoleic Acid (g)	- -				- -	
ALA (g)	- -					
EPA (g)		+				
Very Long Chain Omega-3 Fatty Acids; DHA:EPA:DPA (g)		+				
Vitamin E (mg)	- -				-	
Magnesium (mg)	-				-	
Calcium (mg)					-	
Zinc (mg)	-					
Grains					- -	-
Refined Grains				-	-	
Meat and meat alternatives	-					
Processed meats		+				
Poultry					-	
Nuts						-
Oil Equivalents	-					

+ symbol denotes a positive, significant correlation which is significant at the 0.05 level (2-tailed).
+ + symbol denotes a positive, significant correlation which is significant at the 0.01 level (2-tailed).
- symbol denotes a negative, significant correlation which is significant at the 0.05 level (2-tailed).
- - symbol denotes a positive, significant correlation which is significant at the 0.01 level (2-tailed).

3.4.12 Multiple Regression Analysis

In order to assess the predictive ability of anthropometric, biochemical and dietary intake variables that were significant in the correlation analysis for each primary cytokine and adipokine marker multiple regression analyses were performed; after controlling for age, sex and diabetes status. Variables that did not show any evidence for normal distribution were transformed using logarithm to base 10 (log10) and reciprocal (inverse) techniques. Once transformed, data were normally distributed and input into the regression model.

3.4.12.1 hs-CRP

Multiple (linear) regression analysis was used to assess the ability of two anthropometric variables (BMI, FM (%)) and three dietary variables (cholesterol, energy and total fat intake) to predict hs-CRP, results of this regression model are presented in **Table 3.11**.

Within this regression model, age, sex and diabetes (step 1) accounted for a non-significant 7% variance in hs-CRP, R^2 = 0.07, F (3, 34) = 0.82, p = 0.492. After entry of the anthropometric variables to the regression model (step 2), BMI and fat mass (%) accounted for an additional significant 21% of the variance in hs-CRP, ΔR^2= *0*.21, ΔF (2, 32) = 4.74, p = 0.016. Once the dietary variables were added to the regression model (step 3), cholesterol, energy and total fat intake accounted for an additional 14% of the variance in hs-CRP, ΔR^2= .14, ΔF (3, 29) = 2.32, p = 0.096. The total variance explained by the final model was 42%, R^2 = .42, F (8, 29) = 2.63, p = 0.027. In the final model, the only significant individual predictor of hs-CRP was BMI (β = 0.66, p = 0.024).

Table 3.11. Multiple linear regression model for the outcome variable hs-CRP† at baseline

Model				
hs-CRP	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1 (Confounders)	Age	0.00 [-0.01, 0.01]	0.08	0.67
	Sex	0.06 [-0.30, 0.41]	0.07	0.75
	Diabetes	-0.12 [-0.49, 0.24]	-0.16	0.50
Step 2	Age	0.00 [-0.01, 0.01]	0.14	0.44
	Sex	0.18 [-0.38, 0.74]	0.23	0.52
	Diabetes	0.01 [-0.34, 0.36]	0.01	0.96
	BMI	0.03 [0.00, 0.07]	0.53	0.08
	FM (%)	0.00 [-0.04, 0.03]	-0.09	0.82
Step 3	Age	0.00 [-0.01, 0.01]	0.03	0.85
	Sex	0.19 [-0.34, 0.72]	0.24	0.47
	Diabetes	-0.02 [-0.36, 0.33]	-0.02	0.92
	BMI	0.04 [0.01, 0.08]	0.66	0.024*
	FM (%)	-0.01 [-0.05, 0.02]	-0.31	0.45
	Energy (kJ)†	-0.60 [-2.48, 1.28]	-0.23	0.52
	Total Fat (g)†	0.12 [-1.30, 1.54]	0.06	0.86
	Cholesterol (mg)†	-0.55 [-1.29, 0.19]	-0.30	0.14

hs-CRP, high-sensitivity C-reactive protein; Adj, adjusted; df, degrees of freedom; BMI, body mass index; FM (%), fat mass percent. †Log (base 10)-transformed variable. *Significant, P <0.05.

3.4.12.2 TNF-α

Multiple (linear) regression analysis was utilised to assess the ability of one anthropometric measure (body weight) and one biochemical variable (glucose) to predict TNF-α, results for this regression model are presented in **Table 3.12**.

On step 1, age, sex and diabetes status accounted for a non-significant 10% variance in TNF-α, R^2 = 0.10, F (3, 37) = 1.33, p = 0.280. Body weight and glucose were then entered into the regression model, these variables accounted for an additional significant 26% of the variance in TNF-α, ΔR^2 = .26, ΔF (2, 35) = 7.18, p = 0.002. The total variance explained by the model was 36%, R^2 = 0.36, F (5, 35) = 3.94, p = 0.006. Both predictors of TNF-α were statistically significant and weight (kg) had a greater magnitude of effect (β = 0.47, p = 0.002) than glucose (β = -0.33, p = 0.045).

Table 3.12. Multiple linear regression model for the outcome variable TNF-α† at baseline

Model				
TNF-α	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1	Age	0.00 [-0.01, 0.01]	0.05	0.77
(Confounders)	Sex	-0.19 [-0.40, 0.03]	-0.35	0.09
	Diabetes	-0.18 [-0.40, 0.04]	-0.34	0.10
Step 2	Age	0.00 [0.00, 0.01]	0.12	0.45
	Sex	-0.06 [-0.26, 0.14]	-0.11	0.55
	Diabetes	0.01 [-0.21, 0.23]	0.01	0.96
	Weight (kg)	0.01 [0.00, 0.01]	0.47	0.002*
	Glucose (mmol/L)ǂ	-2.33 [-4.60, -0.05]	-0.33	0.045*

TNF-α, tumor necrosis factor-alpha; Adj, adjusted; df, degrees of freedom. †Log (base 10)-transformed variable. ǂReciprocal (inverse)-transformed variable. *Significant, P <0.05.

3.4.12.3 IL-6

Multiple (linear) regression analysis was used to assess the ability of two dietary measures (PUFAs as % contribution of total energy intake, SFAs as % contribution of total energy intake) to predict serum IL-6, results for this regression model are presented in **Table 3.13**.

Within this regression model, at step one age, sex and diabetes accounted for a non-significant 13% variance in IL-6, R^2 = .13, F (3, 37) = 1.77, p = 0.169. After entry of dietary variables to the regression equation, PUFAs (%E) and SFA (%E) accounted for an additional and significant 19% of the variance in IL-6, ΔR^2 = .19, ΔF (2, 35) = 4.91, p = 0.013. The total variance explained by the whole model was 32%, R^2 = .32, F (5, 35) = 3.26, p = 0.016. In the whole model, only two predictors were statistically significant and of these, sex had a greater magnitude of effect (β = 0.50, p = 0.009) than saturated fat as a percent of total energy intake (β = 0.39, p = 0.018).

Table 3.13. Multiple linear regression model for the outcome variable IL-6† at baseline

Model				
IL-6	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1	Age	0.00 [-0.02, 0.02]	0.01	0.97
(Confounders)	Sex	0.50 [0.01, 0.98]	0.40	0.047*

	Diabetes	0.12 [-0.38, 0.61	0.10	0.63
Step 2	Age	0.00 [-0.01, 0.02]	0.05	0.76
	Sex	0.62 [0.16, 1.07]	0.50	0.009*
	Diabetes	0.20 [-0.26, 0.65]	0.16	0.38
	SFA (%E)	0.07 [0.01, 0.14]	0.39	0.018*
	PUFA (%E)†	-0.40 [-1.51, 0.71]	-0.11	0.47

IL-6, interleukin-6; Adj, adjusted; df, degrees of freedom; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. †Log (base 10)-transformed variable. *Significant, P <0.05.

3.4.12.4 Adiponectin

Multiple (linear) regression analysis was employed to assess the ability of two anthropometric variables (BMI, FM (%)) and two biochemical variables (AST, ALT) to predict levels of adiponectin, and the results of this regression model are presented in **Table 3.14**.

On step 1, age, sex and diabetes status accounted for a non-significant 13% variance in adiponectin, R^2 = .13, F (3, 37) = 1.83, p = 0.160. On step 2, the entry of anthropometric variables BMI and FM (%) to the regression equation, accounted for an additional significant 21% variance in adiponectin, ΔR^2= .21, ΔF (2, 35) = 5.52, p = 0.008. On step 3, the addition of biochemical variables AST and ALT accounted for an additional non-significant 10% of the variance in adiponectin, ΔR^2= .10, ΔF (2, 33) = 2.80, p = 0.075. The total variance explained by the whole model was 43%, R^2= .43, F (7, 33) = 3.61, p = 0.005, however there were no individual statistically significant predictors in this model (p>0.05).

Table 3.14. Multiple linear regression model for the outcome variable Adiponectin† at baseline

Model				
Adiponectin	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1 (Confounders)	Age	0.01 [0.00, 0.02]	0.25	0.15
	Sex	0.16 [-0.09, 0.41]	0.25	0.21
	Diabetes	0.08 [-0.18, 0.33]	0.12	0.55
Step 2	Age	0.01 [0.00, 0.01]	0.24	0.14
	Sex	-0.09 [-0.50, 0.32]	-0.14	0.66
	Diabetes	0.13 [-0.11, 0.37]	0.21	0.27
	BMI	0.00 [-0.03, 0.03]	-0.01	0.96
	FM (%)	0.03 [0.00, 0.06]	0.66	0.08
Step 3	Age	0.01 [0.00, 0.02]	0.33	0.06
	Sex	-0.07 [-0.46, 0.32]	-0.11	0.72
	Diabetes	0.06 [-0.18, 0.30]	0.09	0.63
	BMI	0.00 [-0.03, 0.03]	0.02	0.93
	FM (%)	0.02 [-0.01, 0.05]	0.53	0.14
	ALT (U/L)†	0.66 [-0.11, 1.43]	0.50	0.09
	AST (U/L)†	-0.32 [-1.21, 0.58]	-0.20	0.48

Adj, adjusted; df, degrees of freedom; BMI, body mass index; FM (%), fat mass percent; ALT, alanine aminotransferase; AST, aspartate aminotransferase. †Log (base 10)-transformed variable. *Significant, P <0.05.

3.4.12.5 Resistin

Multiple (linear) regression analysis was completed to assess the ability of two anthropometric variables (BMI, FM (kg)), to predict levels of leptin, results of this regression model are presented in **Table 3.15**.

On step 1 of the regression model, age, sex and diabetes status accounted for a non-significant 3% variance in resistin, R^2 = .03, F (3, 37) = 0.35, p = 0.786. After entry of anthropometric variables, BMI and FM (kg) accounted for an additional non-significant 11% of the variance in resistin, ΔR^2= .11, ΔF (2, 35) = 2.29, p = 0.120. The total variance explained by the whole regression model was 14%, R^2= .14, F (5, 35) = 1.13, p = 0.364. There were no individual statistically significant predictors in this model (p>0.05).

Table 3.15. Multiple linear regression model for the outcome variable Resistin† at baseline

Model				
Resistin	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1	Age	0.00 [-0.01, 0.00]	-0.11	0.55
(Confounders)	Sex	-0.02 [-0.18, 0.14]	-0.06	0.78
	Diabetes	-0.08 [-0.24, 0.09]	-0.21	0.34
Step 2	Age	0.00 [-0.01, 0.00]	-0.08	0.67
	Sex	-0.03 [-0.21, 0.15]	-0.07	0.76
	Diabetes	-0.04 [-0.21, 0.12]	-0.11	0.61
	BMI	0.00 [-0.03, 0.03]	-0.01	0.99
	FM (kg)	0.44 [-0.77, 1.65]	0.35	0.46

Adj, adjusted; df, degrees of freedom; BMI, body mass index; FM (kg), fat mass (kilograms). †Log (base 10)-transformed variable. *Significant, P <0.05.

3.4.12.6 Leptin

Multiple (linear) regression was used to assess the ability of two anthropometric variables (BMI, FM (%)), two biochemical variables (AST, HOMA-IR) and one dietary variable (linoleic acid) to predict levels of leptin. Results of the regression model for leptin are presented in **Table 3.16**.

Within this regression model, age, sex and diabetes status (step 1) accounted for a significant 24% variance in leptin, R^2 = .24, F (3, 37) = 3.97, p = 0.015. At step 2, the anthropometric variables BMI and FM (%) were added to the regression equation which accounted for an additional significant 39% of the variance in leptin, ΔR^2= .39, ΔF (2, 35) = 18.11, p = 0.0005. At step 3, biochemical variables AST and HOMA-IR accounted for an additional non-significant 4% of the variance in

leptin, $\Delta R^2 = .04$, $\Delta F\ (2, 33) = 1.86$, $p = 0.172$. At step 4, the dietary variable linoleic acid accounted for an additional 0.2% of the variance in leptin, $\Delta R^2 = .002$, $\Delta F\ (1, 32) = 0.22$, $p = 0.641$. The total variance explained by the whole regression model was 67%, $R^2 = .67$, $F\ (8, 32) = 8.06$, $p < 0.001$*, with the only significant individual predictor of leptin being diabetes diagnosis ($\beta = 0.45$, $p = 0.016$).

Table 3.16. Multiple linear regression model for the outcome variable Leptin† at baseline

Model				
Leptin	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1 (Confounders)	Age	0.00 [-0.01, 0.01]	0.09	0.60
	Sex	0.36 [0.11, 0.61]	0.53	0.01
	Diabetes	0.09 [-0.17, 0.35]	0.14	0.47
Step 2	Age	0.00 [-0.00, 0.01]	0.11	0.36
	Sex	0.23 [-0.10, 0.56]	0.34	0.16
	Diabetes	0.21 [0.02, 0.40]	0.32	0.033*
	BMI	0.02 [0.00, 0.04]	0.34	0.10
	FM (%)	0.02 [0.00, 0.04]	0.44	0.12
Step 3	Age	0.00 [0.00, 0.01]	0.11	0.34
	Sex	0.31 [-0.03, 0.64]	0.46	0.07
	Diabetes	0.28 [0.06, 0.51]	0.42	0.015*
	BMI	0.02 [0.00, 0.04]	0.38	0.06
	FM (%)	0.01 [-0.01, 0.04]	0.31	0.26
	AST (U/L)†	0.08 [-0.31, 0.47]	0.05	0.69
	HOMA-IR†	0.24 [-0.06, 0.55]	0.21	0.11
Step 4	Age	0.00 [0.00, 0.01]	0.12	0.33
	Sex	0.31 [-0.03, 0.64]	0.46	0.07
	Diabetes	0.30 [0.06, 0.54]	0.45	0.016*
	BMI	0.02 [0.00, 0.04]	0.38	0.07
	FM (%)	0.01 [-0.01, 0.04]	0.34	0.24
	AST (U/L)†	0.08 [-0.32, 0.47]	0.04	0.70
	HOMA-IR†	0.27 [-0.06, 0.60]	0.23	0.11
	Linoleic Acid†	0.08 [-0.25, 0.41]	0.06	0.64

Adj, adjusted; df, degrees of freedom; BMI, body mass index; FM (%), fat mass percent; AST, aspartate aminotransferase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance. †Log (base 10)-transformed variable. *Significant, P <0.05.

3.5 Discussion

The results of this chapter provided a detailed description of the sociodemographic, anthropometric and body composition, biochemistry, inflammatory, liver, and dietary intake profiles of individuals with NAFLD who were recruited for the MEDINA trial, and characterised by sex and criteria for MetS. The results of this cross-sectional analysis indicated that the majority of participants were overweight and obese, insulin resistant and had elevated liver outcomes, reflective of fatty infiltration and moderate/mild fibrosis. Males had particularly low levels of adiponectin and higher VF than females, while females had increased levels of circulating IL-6 and leptin. In the full cohort, adiponectin was positively associated with obesity markers (BMI and fat mass), liver enzymes (ALT and AST) and leptin. The inflammatory marker leptin was significantly correlated with other inflammatory markers hs-CRP, TNF-α and adiponectin. BMI was a strong predictor of hs-CRP; sex (female) and SFA were the strongest predictors of IL-6, and sex and diabetes were strongest predictors of leptin. Baseline dietary intake analysis showed that this group did not meet the population recommendations for energy and carbohydrate intake but were consuming high amounts of total and saturated fats, total sugars and protein. Over half of the cohort fit the criteria for MetS and, as expected, participants with the MetS and NAFLD had significantly higher levels of fasting insulin, HOMA-IR, HDL-c and triglycerides, compared to those without the MetS. There did not appear to be any other significant differences in anthropometry or body composition, biochemical, inflammatory, or liver markers in those with both the MetS and NAFLD in comparison to individuals without the MetS.

Within this NAFLD cohort, over 90% of participants were overweight or obese with body composition measurements exceeding population norms; more than half of the cohort met criteria for the MetS and just under half were diagnosed with T2DM. Participants in the MEDINA study were representative of a wider NAFLD population, as determined by clinical characteristics of the group [407, 408] and overlap of metabolic disorders.[409, 410] As anticipated, participants with the MetS had higher levels of fasting insulin, HOMA-IR and diagnostic markers for the MetS (HDL-c and triglycerides), which suggests the presence of metabolic alterations.[411] Insulin resistance will induce chronic hyperglycaemia, which initiates oxidative stress and triggers an inflammatory response leading to cell death.[412] Marchesini et al. (2003) found that even after controlling for sex, age and body mass, participants with the MetS and NAFLD carried a higher risk of NASH fibrosis.[373] In skeletal muscle and adipose tissue, IR affects glycogen synthesis, protein catabolism and lipoprotein lipase function resulting in an increase of FFAs and release of inflammatory markers including IL-6, TNF-α and leptin.[412] Indeed, IR and inflammation are important clinical implications for the future development of adverse cardiovascular events. In the present cohort, the MetS group had a similar inflammatory profile and IHL (%) to the non-MetS/NAFLD only group. This indicates that NAFLD is indeed the *hepatic manifestation of the MetS*, but also highlights that NAFLD is present in both the MetS and worryingly present in people without these risk factors. A recent observational

study conducted in two Melbourne metropolitan hospitals highlighted the importance of capturing patients with metabolic abnormalities in the clinical setting prior to them progressing to end-stage liver and CVD.[47] Multidisciplinary clinical approaches could be useful to screen and manage high-risk patients with multiple metabolic disorders.

The pro-inflammatory cytokines CRP, TNF-α, IL-6, and adipokines leptin and resistin, were investigated in this study as they have been previously linked to the pathogenesis of NAFLD and progression of fibrosis in NASH.[413] Levels of these inflammatory mediators are not widely reported in a NAFLD or chronic liver disease cohort, therefore it is difficult to determine the severity of inflammation present in this cohort given there were no healthy controls included in the study. A study conducted by Jarrar et al. (2008) aimed to assess levels of circulating cytokine and adipokine markers in obese controls compared to patients with NAFLD and NASH. The inflammatory markers assessed included TNF-α, IL-6, adiponectin and resistin,[173] and in comparison to MEDINA participants in this cohort circulating levels of TNF-α and adiponectin corresponded with classifications ranging between "simple steatosis" and "NAFLD diagnosis" in Jarrar's study group.[173] While circulating levels of adiponectin were low in participants enrolled in the MEDINA trial, adiponectin measures were not markedly lower than healthy populations and were not to the extent of other NAFLD or NASH populations studied (classified as hypoadiponectinemia).[414-416] Circulating IL-6 was considerably higher in their study participants and not comparable to this group, whereas circulating resistin was significantly lower in their group and higher in this group of participants.[173] While resistin has been associated with steatosis and portal inflammation and histological NAS,[415] the role of resistin as an inflammatory marker has only been partly uncovered and its role in the pathogenesis of IR, obesity and NAFLD is still largely unclear.[173] Serum resistin is variable in NAFLD patients and have been found to be higher,[417] lower,[418] or without difference[173, 419] compared with healthy or obese controls. Alternatively, TNF-α was markedly raised in the present study group compared to previously studied NAFLD patients and corresponding healthy controls,[416] whereas IL-6 was found to be lower in this group when compared to the same NAFLD patients and healthy controls.[416] It is well known that proinflammatory cytokines TNF-α and IL-6 are drivers of disease progression in NAFLD,[415] NASH [420, 421] and CVDs,[422] though circulating levels improve with lifestyle modification together with improvement of liver damage.[417, 423] In vivo, accumulating evidence indicates that resistin has strong proinflammatory properties partially stimulating the release of IL-6 and TNF-α.[424] Thus, it is possible that a participating pathway for cytokine release may be activated by the underlying mechanisms of resistin leading to NAFLD and associated liver damage.[424, 425]

Adiponectin is the only known adipokine that is down-regulated in obesity and is therefore thought to have a protective, anti-inflammatory and anti-fibrotic potential. The results of a regression analysis in the present study found that BMI and fat mass (%) were positive, significant predictors for high levels of circulating adiponectin. One study investigating patients with NAFLD, chronic

hepatitis B (CHB) and healthy controls found similar adiponectin levels to this cohort for those diagnosed with CHB or healthy controls, though their NAFLD patients were slightly more adiponectin deficient.[426] [427] Another study in NAFLD patients, obese controls and healthy controls saw altered (low) levels of serum adiponectin in those with NAFLD and higher levels in both obese and healthy controls.[417] The present MEDINA groups levels of adiponectin were in comparison with that of the obese control participants in their study. The results of the present study showed that circulating levels of adiponectin were higher than that of other NAFLD cohorts, which could also be indicative of a less severe disease phenotype observed in the MEDINA study.[428, 429]A systematic review and meta-analysis which included twenty-seven studies found that although total serum adiponectin was higher in controls compared with NAFLD or NASH patients, adiponectin was similar between controls and NAFLD patients when controls were subjected to liver biopsy.[421] Authors speculated that higher adiponectin was associated with no or milder liver injury meaning that hypoadiponectinemia plays a role in the progression from NAFLD to NASH (secondary hit), whereas its role in the development of NAFLD (first hit), if any, is yet to be defined.[421] Although most research eludes to the anti-inflammatory potential of adiponectin, some epidemiological studies have observed similar positive associations between adiponectin, increased cardiovascular risk and mortality in obese, diseased populations.[427, 430-432] One prospective study reported a positive association between high plasma adiponectin levels and mortality due to chronic heart failure, also associated with a high BMI.[433] Meanwhile, Pilz et al. (2006) found that high adiponectin independently predicted all cause, cardiovascular and non-cardiovascular mortality.[434] NAFLD is considered a key precursor to CVD-related mortality. Since not all studies agree with the complex nature of adiponectin and its various isoforms, more studies are required to form firm conclusions.

In this cohort, females tended to have a higher inflammatory profile than men for all pro-inflammatory cytokine and adipokine markers, particularly in leptin and adiponectin. Of interest, the regression model for leptin found that combining age, sex (female over male), diabetes diagnosis, and BMI and fat mass (%), were all significant predictors of leptin. Additionally, the presence of diabetes was an independent predictor of leptin. In 2009, Argentou et al.[435] also found a positive association between serum leptin and the presence of diabetes in obese patients with NAFLD. Furthermore, their study showed a trend for association of leptin with percentage body fat as seen in this study.[435] It was also noteworthy that the females in this cohort had a less favourable body composition with higher rates of adiposity and intrahepatic lipids, and a greater diagnosis of T2DM and IR. Similarly, Westerbacka et al. (2004) found that women tended to have higher levels of subcutaneous fat, liver fat and fasting insulin, though men had more intraabdominal (or visceral) fat than females.[436] They also found that this excess intraabdominal fat was linked to adiponectin deficiency in men,[436] this too was seen in this cohort's male population. Males in this cohort tended to have significantly more visceral fat and notably lower levels of circulating adiponectin. Previous literature indicate that males tend to have higher amounts of visceral fat, lower levels of adiponectin and higher levels of TNF-α due to the functional differences in visceral adipose versus subcutaneous

tissue.[437] These findings are in keeping with previous studies, which interestingly reported that when matched for age, BMI and insulin sensitivity, both adiponectin and leptin levels are greater in females.[438] Argentou et al. (2009) also noted sex differences in serum leptin and adiponectin levels which were both higher in women compared to men,[435] further supporting previous studies in chronic liver disease and highlighting sex-related differences in circulating adipokine levels.[439] Since numerous physiological factors can affect circulating levels of adiponectin, including age, sex, insulin sensitivity and body fat distribution, it is difficult to evaluate the clinical significance of such a sensitive marker of disease. Together, these studies highlight the importance of categorising pro-inflammatory and anti-inflammatory markers with any important disease variables in order to assess progression and severity of liver disease.

Sex differences apparent in NAFLD have been associated with adipose tissue and adipocytokine distribution, but also with interactions between sex hormones and insulin resistance.[440] Adipocytes found in subcutaneous adipose tissue are more dense in oestrogen receptors and leptin release, which can also protect against visceral adipose tissue accumulation.[440] It is well known that sex and age exhibit effects on disease prevalence and severity. Sex and age are both major influential factors in the compartmentalization of adipose tissue, and females in this study were also significantly older than males. Among younger patients, NAFLD and NASH is more common in men (2–3 times); however, after the age of 60 (y), the prevalence of NASH is higher in women.[441, 442] Age and sex differences imply that oestrogen may play a protective pathophysiological role in NAFLD, however additional studies are required to explain this association.[443]

There were slightly more females than males in this study group and the majority of patients were of Asian ethnicity. The prevalence of NAFLD is rising at alarming rates in Asian populations due to the reported genetic predisposition to NAFLD and NASH for those of Asian ethnicity.[71, 444-446] Rates of NAFLD prevalence are now reported to be as high as 20% in China, 27% in Hong Kong, and 15–45% in South Asia, South-East Asia, Korea, Japan and Taiwan.[444, 447-450] Studies suggest the effects of the increasingly common change to a more Western-style dietary pattern and increased sedentary lifestyle are associated with the increase in prevalence of obesity and chronic diet-related disease in Asian countries.[451] Aside from poor diet, susceptibly to NAFLD is considered as inherited, after various genome-wide association studies have indicated that several single nucleotide polymorphisms (SNPs) affect NAFLD prevalence and severity.[444] Certain genotypes in ethnic populations have been associated with a higher risk for developing T2DM, NAFLD and NASH.[442]

Regression models for hs-CRP, TNF-α and IL-6 were significant, however the model for resistin was not significant. BMI was a significant predictor of hs-CRP, a strong association which has been well-reported in the literature.[155, 452-454] CRP was thought to be only produced by the liver, though these associations indicate that adipose tissue may also induce the up-regulation of CRP production. TNF-α was significantly predicted by body weight and glucose. TNF-α has been previously

associated with measures of visceral adiposity and is often implicated in the development of obesity and IR, hence the results of this study support this implication.[455, 456] It has also been reported that TNF-α should be investigated in large populations for significant associations to be seen,[456] therefore the sample size of this study may have been inadequate to detect significant associations between other variables such as IR and VF. Sex and saturated fat as a percentage of total energy were significant predictors of IL-6, a finding which has not been widely reported in previous literature. The accumulation of FFAs in hepatocytes may indeed be a result of increased dietary intake of SFAs, and this accumulation activates IKK-B and NF-κB transcription factors which are the pathways for release of various proinflammatory makers including IL-6.[456] The positive relationship between IL-6 and SFA may be attributed to this mechanism. In addition to these findings, leptin was significantly associated with hs-CRP, TNF-α and adiponectin. Interestingly, elevated levels of leptin have previously been associated with enhanced levels of certain inflammatory species including IL-1 and TNF-α. In this cycle, levels of circulating serum leptin and other cytokines feed the prolonged status of a pro-inflammatory milieu.[457, 458] The positive association and ability for leptin to predict adiponectin may be related to respective roles in glucose metabolism, however given future studies are needed to determine the role and association inflammation and leptin secretion in NAFLD patients.

Liver biochemistry, ^{1}H-MRS (IHL%) and TE (LSM) were used to measure liver health. ALT was elevated in this cohort, though this was required for participation in the study (as listed in the aforementioned eligibility criteria). This NAFLD cohort had liver enzymes within the normal ranges except for GGT (which was above the reference range for a healthy population), increased IHL (%) and increased LSM. There were no differences in liver outcomes and histology between male and female participants, or between MetS or non-MetS participants. Furthermore, only adiponectin was significantly and moderately associated with ALT and AST. Elevated aminotransferase levels (ALT/AST) are often indicative of hepatocellular injury, and the alkaline phosphatase and bilirubin tend to be elevated in cholestatic injury.[375] Studies in similar populations have shown that although elevated ALT is routinely used as an entry criterion, values of other liver enzymes, AST, ALP and GGT, often remain unchanged in study groups and the low sensitivity of these tests make it difficult to differentiate between healthy controls and a NAFLD population via these markers.[243, 459] Alternatively, liver enzymes are seen to be significantly elevated in progressed liver diseases, including NASH and liver cirrhosis.[399] Though liver enzymes were previously considered a strategy for NAFLD diagnosis and management, many studies have now deemed these liver biochemistries a non-effective and non-precise indication of fatty liver.[243, 399, 459] Increasingly sensitive, precise and non-invasive markers are required to give a true indication of prevalence or severity of liver disease and liver histology.[345] The usefulness of serum markers of inflammation as a general diagnostic and monitoring method is widely being explored.[460]

Accurately assessing dietary intake in patients in the real world setting is difficult and susceptible to error.[395] Variability in dietary composition data and under-reporting of food and/or beverage intake often occurs where self-reported diet data is collected from participants. Dietary intake within this cohort was self-reported and thoroughly checked by a dietitian, though the potential for under-reporting either due to inability to recall diet history or the tendency to alter reporting in the presence of a nutrition professional could have occurred as a consequence of this methodology. Nevertheless, despite a low total energy and carbohydrate intake, participants in this cohort were consuming higher amounts of total fat, sugars and protein. In comparison to RDI's, this cohort had a high intake of SFA and low intake of PUFAs. Similar to this, Cortez-Pinto and colleagues identified significantly lower carbohydrate, and higher total fat and PUFA consumption in NAFLD patients compared with healthy controls.[461] Cortez-Pinto and colleagues also found that their participants were consuming higher amounts of MUFAs in comparison to SFAs, and these were both above the recommended intakes, however consumption of PUFAs fell below the recommended intake. These results were reflective of our study groups fat intake pattern which is noteworthy considering the strong causal link between excess fat consumption, obesity and NAFLD. Ideally, diets should contain <10% SFA in order to alleviate unfavourable changes in IR and triglyceride content.[462]

As previously mentioned, although total carbohydrate intake was lower than the recommended intake, total protein and sugar consumption was increased in this population. Indeed, the harmful effects of excess sugar intake on hepatic and metabolic functions contributing to NAFLD pathogenesis have been widely reported. So too, have the effects of animal-derived protein intake on liver damage.[463] Zelber-Sagi et al.[464] conducted a cross-sectional study in NAFLD patients living in Israel, and found that their patients were consuming higher amounts of sugar (particularly soft drinks), meat protein and lower intakes of fish rich in omega-3. A smaller study in Japan reported that a group with NASH were consuming higher amounts of simple carbohydrates, glucose and fructose, in the form of fruit, confectioneries, sweetened cereals, and cookies, than patients in their NAFLD group.[465] They did find, however, that their NAFLD patients consumed higher amounts of protein and zinc compared to the NASH patients. Researchers concluded that foods high in sugar, and therefore glycaemic index (GI), stimulate excessive and prolonged insulin secretion, resulting in increased fat mass and non-esterified free fatty acid circulation, which are then deposited for accumulation as lipids in hepatocytes of the liver.[465] Essentially, this is the "first hit" in the pathogenesis of NAFLD.

Participants were consuming lower than the recommended serves of wholegrains and higher than the recommended serves of refined grains, indicative of poor diet quality typically seen in a NAFLD population. The type and amount of carbohydrates have been implicated in the aetiology of chronic diseases such as obesity, diabetes and dyslipidaemia, affecting body weight and adiposity directly.[465] Altogether, the complex nature of wholegrains are protective and rich in compounds that function as antioxidants, such as trace minerals and phenolic compounds, and phytoestrogens, with

potential hormonal effects.[466] Wholegrains have received considerable attention recently in NAFLD, particularly within a Mediterranean Dietary pattern in which it is a staple food item and has been linked to a decrease in hepatic steatosis and improved IR.[467] A wider range of studies incorporating wholegrains into a well-balanced dietary pattern are warranted in the context of NAFLD management.

A surprising observation in this cohort was the adequate intake of dietary fibre, considering the under-consumption of wholegrains, fruit and vegetable servings. An in-depth analysis into the types of fruits and vegetables being consumed was not completed, although participants may have been consuming staple foods high in fibre even though overall intake was inadequate. Fruit and vegetable intake were particularly low in this cohort, which is often observed in obese populations.[468] Similarly, a study in Japan reported inadequate intake of vegetables in NAFLD patients compared with T2DM patients.[469] Also staple foods in the Mediterranean Diet, vegetables and fruits are considered to have a pivotal role in preventing the oxidative stress process,[470] while encompassing components such as antioxidants, phytosterols and phytochemicals reducing CVD risk.[471]

A strength of this study was that the dietary intake data for this cohort was descriptive and cross checked by a dietitian within the participant's dietary consultation to ensure that the food diary was completed thoroughly. Another strength of this study was the extensive medical documentation provided to researchers through each hospital clinic database, including existing co-morbidities and follow-up documentation. Biochemistry and liver enzyme profiles were tested at the Alfred Hospital Pathology Clinic providing accuracy, and ^{1}H-MRS is the gold-standard measurement of IHL (%). Liver histology was assessed using TE, Fibroscan and although this is a common method of fibrosis measurement, it can sometimes be inaccurate in an obese population. Limitations of this study included participant over- or under-reporting due to monitoring can cause deviations and inaccuracies in the data resulting in false meeting of nutritional targets. Typically cross-sectional studies will contain a larger sample size than that which was studied in the MEDINA Trial, however recruitment into the trial was slow due to the eligibility criteria within the trial and the small numbers of NAFLD patients referred to and captured in the clinical setting.[47] The smaller study group result in less powerful statistical analyses and fewer significant associations, it also allows for the sex difference (more females recruited than males) to be more predominant in nature. Future studies are warranted in larger NAFLD populations in order to better identify and describe characteristics of liver injury, inflammation and dietary intake in these patients.

3.5.1 Conclusion

In conclusion, participants enrolled in the MEDINA trial were overweight and insulin resistant at baseline. The anthropometric profile of participants was similar to other cohorts of patients with NAFLD, in terms of increased body mass and adiposity. Although some metabolic biomarkers, inflammatory markers and liver outcomes were elevated to the upper-limit of a healthy or "normal" range, these participants could be classified as metabolically "at-risk" or early stages of NAFLD. There were marked differences in the sex distribution of age, visceral fat and some inflammatory markers in this population and female sex was a predictor of leptin and IL-6. This is indicative of influential mechanisms of sex in individuals with NAFLD and potentially a disparity in disease progression between males and females. Aside from cardiometabolic markers which reflected the diagnostic criteria for the MetS, there were no significant differences in biochemical or inflammatory markers, nor liver outcomes for participants with the MetS in comparison to those without the MetS. This cohort were consuming excess amounts of total and saturated fat, total sugars and (animal) protein and inadequate servings of fruits and vegetables at baseline which is similar to the dietary intake observed in other NAFLD populations. This group was however, consuming an inadequate amount of total energy and carbohydrate. Whether this was an error of under-reporting or a true indication of this cohort's diet composition is unknown and a limitation of the study methodology. Although this cross-sectional study contained some limitations, a descriptive analysis of the MEDINA Study cohort provides an insight into an Australian-based multiethnic NAFLD population. Dietary intervention strategies focussing on anti-inflammatory, insulin-lowering approaches not intended to reduce body weight could be beneficial in this population.

4 The effect of a Mediterranean Diet versus Low-Fat Diet on Inflammation in patients with Non-Alcoholic Fatty Liver Disease: the MEDINA Randomised Controlled Trial

4.1 Abstract

Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) is characterised by the abnormal accumulation of hepatic triglyceride in the liver. The pathogenesis of NAFLD is complex and involves "multiple-hits" prompted by poor diet and environment, leading to insulin resistance (IR), increased visceral adiposity, chronic low-grade inflammation, oxidative stress and alterations in the gut microbiome. Diet and lifestyle interventions remain the primary management strategy in the treatment of NAFLD. The Mediterranean diet (MedDiet) is recognised for lowering hepatic steatosis, IR and liver enzymes in patients with diabetes and the metabolic syndrome, in part, via its anti-inflammatory properties. The **primary aim** of this study was to determine the effect of an *ad libitum* MedDiet versus low-fat diet on inflammatory markers in Australian patients with NAFLD. Secondary aims were to assess the effect of dietary intervention on liver outcomes, insulin resistance, biochemistry, and anthropometry in patients with NAFLD.

Methods: Forty-two patients with biopsy or ultrasound proven NAFLD were randomised to the MedDiet (n=19) or low-fat diet (n=23). Participants attended dietary consultations at 0-, 6- and 12-weeks and measurements of anthropometry, body composition, blood samples and liver outcomes were taken at these timepoints. Diet data was collected using 3-day food diaries and validated 14-item MedDiet or low-fat diet adherence questionnaires.

Results: Thirty nine participants completed the intervention, MedDiet (n=18) and low-fat diet (n=21). The MedDiet group significantly improved levels of adiponectin from baseline to 12-weeks (13.7 ± 9.2µg/mL to 17.0 ± 12.5µg/mL, p =0.016). Significant reductions in fasting insulin and HOMA-IR were seen in the LFD group (20.0 ± 12.4mIU/L to 16.4 ± 11.3mIU/L, p =0.031, and 6.5 ± 5.6 to 5.5 ± 5.5, p =0.047, respectively). No inflammatory markers improved following the LFD. Mean weight change in the LFD group was -4.02 ± 2.29kg, albeit not significant (p >0.05). Both diet groups experienced a significant reduction in VF content from baseline to end-intervention, the reduction was significantly greater in the LFD group compared to the MedDiet group (4.4 ± 2.1L to 1.5 ± 0.6L, p =0.0005, vs 3.2 ± 1.5L to 1.8 ± 1.4L, p =0.0005). Diet quality and adherence to a Mediterranean-style dietary pattern significantly improved for all participants, regardless of diet group assignment.

Conclusion: This study found that adherence to the MedDiet significantly improved the anti-inflammatory marker adiponectin and visceral fat, in the absence of weight loss. Conversely, the low-fat diet elicited improvements in liver enzymes and insulin resistance, with a marked albeit non-significant reduction in body weight.

4.2 Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of excess lipids in the liver, mostly in the form of triglycerides, and where alcohol consumption is absent (ethanol intake ≤30g/day for men and ≤20g/day for women).[467] In its simplest form, NAFLD occurs as hepatic triglyceride accumulation (or simple steatosis) and as a result of additional inflammation and tissue injury can progress to non-alcoholic steatohepatitis (NASH), with or without fibrosis.[472] Without management, NASH may further progress to cirrhosis and hepatocellular cancer (HCC), increasing the burden of liver transplantation and risk of cardiovascular diseases (CVDs) and all-cause mortality.[473-475] NAFLD has quickly emerged as the most common liver disease in the developed world,[37] paralleling the increase in rates in metabolic diseases such as obesity, type 2 diabetes mellitus (T2DM) and the Metabolic Syndrome (MetS),[476, 477] all known risk factors for the development of CVD.[478] In fact, research has found that NAFLD is a predictor of development of incident T2DM and MetS over a median 5-year follow up.[123] Furthermore, there is an approximately 2-fold increase in cardiovascular events for patients diagnosed with both NAFLD and T2DM.[479]

More recently, NAFLD has been referred to as a "multisystem" disease resulting from a complex interaction of metabolic 'hits', genetic and environmental factors,[480] and affecting several extra-hepatic organs and regulatory pathways.[39] The driving pathogenic mechanisms of NAFLD remain unclear, though theories such as the "multiple-hits model" provide some insight into the various interactions involved. The proposed model identifies 'hits' including diet, insulin resistance (IR), visceral adiposity, a pro-inflammatory state, oxidative stress, alterations in the gut microbiome, genetic predisposition and environmental factors to be primarily responsible for NAFLD development and progression.[467, 481] Excess energy consumption usually in the form of processed foods high in saturated fat, cholesterol and/or fructose often result in increased serum levels of free fatty acids (FFAs), elevates triglycerides and alterations to the gut microbiota.[124, 246] High saturated fat intake leads to ceramide (FFA) synthesis and induces IR, inflammation and cell death in the liver.[482] Alongside a suboptimal diet, physical inactivity and obesity contribute to the storage of fat as visceral adipose tissue.[481] IR also affects adipose tissue, rapidly promoting adipocyte proliferation and dysfunction which then induces lipolysis and the release of pro-inflammatory cytokines and adipokines such as Tumour Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6) and Leptin. Inflammatory cytokines and adipokines play a key role in the progression from steatosis to NASH, while IR amplifies *de novo* lipogenesis (DNL), together sustaining a chronic low-grade inflammatory and insulin resistant state. As a result of these processes, increased hepatic uptake of FFAs and altered activity of the gut microbiota lead to lipotoxicity and mitochondrial dysfunction with oxidative stress, producing reactive oxygen species and endoplasmic reticulum stress with activation of unfolded protein response, all advancing hepatic inflammation.[481]

The interplay between inflammation, IR and obesity in NAFLD has been termed a 'vicious cycle', in which each condition promotes the other and accelerates the development of NAFLD.[124] In the

presence of lipotoxicity and altered adipose tissue function, the activation of several pathways lead to an increase in cytokine and adipokine production and secretion in the liver. Following a high-fat diet, an increase in hepatic activities of nuclear factor κB (NF-κB) was associated with activation of Kupffer cells and increased hepatic expression of interleukin-1 beta (IL-1β), IL-6 and TNF-α.[128] The up-regulation of IL-6 and TNF-α are responsible for an increase in the production of acute phase proteins such as CRP and fibrinogen.[483, 484] Elevated plasma CRP is a well-known marker of inflammation and has been positively associated with risk of CVD.[484]. A number of adipocyte-derived inflammatory markers including adiponectin, leptin and resistin are also associated with NAFLD, predominately released from visceral adipose tissue.[485] Adiponectin is an important mediator in the pathogenesis of NAFLD, exerting favourable metabolic effects and acting as an insulin sensitizer.[486] Hence, lower circulating levels of adiponectin have been found in those with NAFLD.[487] Unlike adiponectin, adipokines leptin and resistin exert proinflammatory,[488, 489] profibrogenic[490] and prodiabetogenic[491, 492] effects on the body. While lipid accumulation is the main initial step in the development of NAFLD, it is IR and a prolonged pro-inflammatory milieu that are thought to drive disease progression. Meanwhile, unhealthy dietary patterns support an inflammatory milieu by increasing the production and release of cytokines (IL-6, TNF-α, CRP) and adipokines (leptin, resistin), while lowering concentrations of circulating adiponectin.[493]

Diet and lifestyle interventions remain the chief management strategy for the treatment of NAFLD in the lack of proven safe and effective pharmacotherapy.[494] Traditionally dietary recommendations for NAFLD included calorie-restricted diets centred around a weight loss of approximately 7-10% body weight.[494] Such diets were based on the theory that a reduction in body weight is directly associated with a reduction in intrahepatic lipid (IHL) through the restriction of excess calories and dietary fats and exercise. Weight loss is also proposed to change the activity of adipose tissue, reduce intra-abdominal adiposity and reverse detrimental inflammatory effects, associated with a decrease in inflammatory markers. A recent systematic review of eight intervention studies in individuals with NAFLD found that diet and lifestyle regimes focussing on energy-restriction and exercise had the most significant improvements in IR and NAFLD severity.[495] Authors of the review did not report on the effects on inflammatory markers, however studies summarised in the systematic review conducted by the Doctoral Candidate [256], found that diets resulting in weight loss (usually calorie-restricted diets) were most effective in reducing inflammatory markers in NAFLD cohorts. In more severe cases of NAFLD and NASH fibrosis however, rapid weight loss may aggravate the degree of fibrosis and inflammation present.[496] A main concern of caloric restriction in this patient group is that the sustainability of weight-loss and associated liver, metabolic and inflammatory outcomes is seldom reported. Weight loss-centred dietary interventions have been effective for short-term improvements in NAFLD, though there remains a lack of consistent high-quality literature concerning the duration, sustainability and robustness of interventions, preventing translation into clinical practice.[495] While weight loss remains a primary outcome in the treatment

of patients with NAFLD, improving diet quality and nutrient profiles independent of weight has become an increasingly recognised treatment approach.[497]

Considering the multifactorial origin of NAFLD, health benefits of strategies or interventions that tackle the pathogenic 'hits' of NAFLD are of increasing interest.[285] The usefulness of the Mediterranean Diet (MedDiet) in lowering hepatic steatosis, IR and liver enzymes independent of weight loss has previously been investigated in patients diagnosed with T2DM and NAFLD.[275, 498, 499] A cross-sectional study by Kontogianni et al. (2014)[500] found that greater adherence to a MedDiet pattern was inversely associated with severity of hepatic steatosis, IR and circulating IL-6 in individuals diagnosed with NASH, to a greater extent than those with NAFLD or simple steatosis. One randomised controlled trial (RCT) conducted in patients with NAFLD found that levels of adiponectin improved whilst adhering to a MedDiet supplemented with polyunsaturated fatty acid (PUFA) enriched olive oil over a duration of 12-months.[380] Currently, there are few well-designed RCTs assessing the impact of MedDiet on inflammatory markers in NAFLD, though the efficacy of a MedDiet in improving inflammatory markers has been evaluated in obese, MetS and high CVD-risk populations.[501-503]

In a randomised-single blind trial of one hundred twenty premenopausal obese women, half were assigned to a low-calorie Mediterranean-style diet with increased physical activity and nutritionist-guided sessions about how to achieve ≥10% reduction in body weight and the other half assigned to a control group, only receiving general information about healthy food choices and exercise.[502] After the 2-year intervention, significant improvements in body weight, IR, IL-6, CRP and adiponectin were seen in the intervention group.[502] The control group also experienced significant reductions in body weight and IR but not in inflammatory markers, leading authors to state that a low-calorie Mediterranean diet with exercise is feasible and sustainable for improving inflammation and IR.[502] The same researchers conducted a study to assess whether a Mediterranean-style diet versus prudent diet affected endothelial function and vascular inflammation in one hundred eighty patients with the MetS.[501] Patients following a Mediterranean-style diet showed greater weight loss and reductions in IR, hs-CRP, IL-6, IL-7 and IL-18 than patients in the prudent diet group.[501] The effect of these changes persisted after adjusting for weight loss, highlighting that the anti-inflammatory effects of the Mediterranean Diet may extend beyond weight loss.[501]

The Mediterranean Diet has received considerable attention for the effects on vascular inflammation in high CVD-risk patient groups. Findings from well-known PREDIMED (*Prevención con Dieta Mediterránea;* Prevention with the Mediterranean Diet) study in patients at high-risk for CVD, showed that a 3-month Mediterranean Diet supplemented with extra virgin olive oil (EVOO) decreased hs-CRP to a greater extent than a Mediterranean Diet supplemented with nuts or a low-fat diet.[503] A study involving patients with diabetes or ≥3 CVD-risk factors observed significant decreases in vascular cellular adhesion molecule-1 and circulating concentrations of IL-6 following a MedDiet supplemented with virgin olive oil or a MedDiet supplemented with nuts, but not

following a low-fat diet.[504] In patients with T2DM, a moderate to high adherence to a Mediterranean-type diet has been associated with lower levels of circulating TNF-α and higher plasma adiponectin concentrations than those with low adherence.[505, 506] Conversely, a recent systematic review assessing the effects of Mediterranean-type diets on inflammatory markers in patients with coronary heart disease found that while observational studies consistently demonstrated an inverse relationship between the MedDiet and inflammatory markers in this population this trend was not confirmed by RCTs, highlighting the paucity of literature in this area.[507] Authors made a crucial note about components of Mediterranean diets in their review, stating that while some trials used what may be considered a 'healthy' diet, they were not necessarily reflective of a traditional Mediterranean dietary pattern which may play a role in the lack of anti-inflammatory effects observed.[507]

The traditional MedDiet is considered a relatively high-fat diet, with principle components including a high consumption of plant-based foods – fruits and vegetables – wholegrains, legumes and nuts, and extra virgin olive oil (EVOO)) as the main source of dietary fat. Fish (including oily fish) is promoted as the main source of protein, with a moderate consumption of poultry, eggs and fermented dairy products and low consumption of red meat and sweets. Moderate consumption of red wine is recommended and during mealtimes. The MedDiet does not promote weight loss but rather provides an abundance of nutrients and anti-inflammatory compounds such as polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs), fibre, low-glycaemic index (GI) carbohydrates and polyphenols.[285] Adherence to this dietary pattern has been associated with a decrease in hs-CRP, IL-6, IL-18, TNF-α and circulating levels of free radicals.[508] The mechanistic feature of EVOO as the main dietary fat allows ingested MUFAs (oleic acid) to decrease oxidized low-density lipoprotein (LDL), LDL-cholesterol and triglyceride (TG) concentration without the concomitant decrease in HDL.[509] Daily consumption of EVOO, but not refined olive oil, was found to be effective in reducing CRP and IL-6 in patients with coronary heart disease.[510] Additionally, the replacement of carbohydrate and saturated fatty acids (SFAs) in the diet with an increase in MUFAs may reduce blood glucose levels and increase in HDL.[511] From another mechanistic standpoint, an increased intake of omega-3 (n-3) PUFAs from oily fish in the MedDiet exerts beneficial, anti-inflammatory effects which upregulate hepatic lipolysis.[512] Indeed, a few studies have shown diets enriched with n-3 PUFAs are able to reduce hepatic steatosis, circulating TNF-α and IR.[380, 381] Polyphenols have been associated with favourable changes in post-prandial dysmetabolism which may reduce levels of CRP.[493, 513] Meanwhile, low-GI carbohydrates rich in fibre are known to decrease glucose absorption, hepatic influx of glucose and DNL, whilst also modulating gut microbiota.[467, 514] A reduction in IHL content and CRP synthesis has also been observed in the context of low-GI diets.[515, 516] In the treatment of chronic disease caused by underlying chronic low-grade inflammation, emphasis is increasingly placed on diets such as the MedDiet that are rich in plant-based foods and recommend avoidance of highly processed, energy-dense food products.[493]

Although the role of specific foods, food groups and individual nutrients are acknowledged in the link between diet and disease, increasing importance is now placed on the evaluation of diet quality and overall food patterns which consider the synergistic effects of nutrients.[493] Several dietary indices have been formed and validated to monitor adherence, adequacy and balance of healthy eating patterns. These dietary indices and scores are evolving to include lifestyle and behavioural factors which may influence an individual's adherence or non-adherence to the dietary pattern of interest.[246] The original Mediterranean Diet Score (MDS) was developed by Trichopoulou et al. (1995) consisting of eight core components of a traditional MedDiet and frequency of food or food group consumption.[517] The PREDIMED group developed a comprehensive 14-point MedDiet questionnaire, which was validated against FFQ data and incorporated Mediterranean cooking style, eating patterns and ingredients.[360] While there is consistent epidemiological evidence of the protective effect of the MedDiet pattern in metabolic and high CVD-risk disorders,[518-521] the effect of improved diet quality and/or adherence to a Mediterranean-style diet on concomitant markers of inflammation in NAFLD is lacking. Moreover, investigating the compounded effects of diet on multiple disease states such as NAFLD and T2DM, may provide investigators with a more comprehensive and well-rounded approach to preventing and managing diseases which develop from the same metabolic abnormalities and inflammatory processes. The assessment of dietary patterns, inflammation and NAFLD in the same patient population throughout a well-designed dietary intervention trial is needed.

4.3 Section One: The effect of a Mediterranean Diet versus a Low-Fat Diet on inflammation in NAFLD

4.3.1 Aims

The **primary aim** of this study was to determine the effect of an *ad libitum* MedDiet versus low-fat diet on inflammatory markers in Australian patients with NAFLD.

The secondary aims for the first section of this research chapter were:

(i) To assess the effect of the MedDiet versus LFD on liver outcomes, insulin resistance, biochemistry, and anthropometry in patients with NAFLD.

(ii) To assess the change in nutrient and food group intake in MedDiet and LFD study groups and compare dietary intake of participants to the prescribed diets.

(iii) To determine the correlation between inflammatory markers and liver, clinical and dietary outcome measures in each diet group.

(iv) To assess change in diet quality and compliance to a MedDiet in a pooled NAFLD cohort and explore differences in key outcomes between higher and lower levels of compliance.

(v) To determine which clinical markers predicted a change in inflammatory status between baseline and end of intervention, regardless of dietary prescription (pooled cohort regression analysis).

4.3.2 Hypotheses:

It was hypothesised that a MedDiet would be beneficial in improving inflammation, liver outcomes and IR in patients with NAFLD. Adherence to key dietary components of a MedDiet pattern may be associated with changes in inflammatory markers and provide insight into a dietary management approach targeting chronic low-grade inflammation rather than weight loss in this population.

4.4 Section Two – Subgroup Analysis:

Assessing the impact of a dietary intervention and overall improved diet quality in patients diagnosed with NAFLD and T2DM (n=18).

4.4.1 Rationale:

Section one of this chapter found that there were differences in the number of participants with type 2 diabetes mellitus randomised to each dietary intervention group. For this reason, further analysis (section two) was conducted in the subset of the population diagnosed with T2DM versus those without T2DM in order to assess their responsiveness to diet.

4.4.2 Aims

The aims of the second section of this research chapter were:

(i) To assess the effect of the MedDiet versus LFD on inflammatory markers, insulin resistance, biomarkers and liver outcomes in a subset of the population of individuals diagnosed with T2DM and NAFLD.

(ii) To assess the impact of overall improved diet quality in individuals diagnosed with T2DM and NAFLD (pooled cohort).

4.4.3 Hypotheses:

It was hypothesised that individuals diagnosed with T2DM and NAFLD may have a greater response to a MedDiet and a LFD intervention, resulting in greater improvements in inflammation, liver outcomes and IR for patients with NAFLD and T2DM versus patients with NAFLD only.

4.5 Methods

4.5.1 Study Design

This study was a multicentre, randomised dietary intervention trial in patients with NAFLD, registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) Trial ID: ACTRN12615001010583. The intervention period ran for 12-weeks, with data collection timepoints at 0-weeks (baseline), 6-weeks (mid-intervention) and 12-weeks (end-intervention). The overarching trial (MEDINA) also had data collection timepoints at 6-months and 12-months in order to assess sustainability of each diet in the study population after the intervention period was complete, however this 6- and 12-month data is not presented in this chapter or doctoral thesis. The protocol for the overarching randomised controlled trial has previously been published by Papamiltiadous et al., (2016),[345] was designed in accordance with and adheres to the Consolidated Standards of Reporting Trials (CONSORT) statement (**Appendix 10**).[522]

4.5.2 Participant Screening and Recruitment

Participants were recruited from the outpatient Liver Clinics of four major metropolitan hospitals across Melbourne: Alfred Health, Eastern Health, The Royal Melbourne Hospital and St Vincent's Hospital. Recruitment was conducted between December 2014 and March 2018. Prior to each liver clinic, patient lists were screened by a trained researcher for potentially eligible participants and the managing hepatologist was informed. The same trained researcher also attended each liver clinic and subsequent to referral from the hepatologist, informed the patient of the trial in lay terms. If the patient was agreeable, a participant informed consent form (PICF) was provided and additional screening was conducted **(Appendix 3.1)**.

4.5.3 Eligibility Criteria

The full eligibility criteria for the MEDINA trial are provided in **Chapter 2, Table 2.1**. Upon fulfilment of this criteria, an additional screening questionnaire (**Appendix 3.2**) was administered to gain further information on the main elements of the eligibility criteria. Briefly, details pertaining to NAFLD diagnosis and imaging were obtained in the form of ultrasound and/or liver biopsy and serum alanine aminotransferase (ALT) levels within the previous 12-months were recorded. Serum ALT were required to be raised (>30 males, >20 females) and <5 times the upper limit of normal (ULN), fasting glucose and insulin levels were collected and homeostatic model assessment-insulin resistance (HOMA-IR) was calculated and required to be >2 units. Medical history and co-morbidities were checked and participants were excluded if they: had a HbA1C level above 8%, had cardiovascular, cerebrovascular and/or peripheral vascular disease, had pulmonary, gastrointestinal, renal, metabolic, haematological, neurological, psychiatric, systemic or any acute infectious disease or signs of acute illness, or psychosocial or gastrointestinal malabsorptive conditions, or currently/previously had bulimia nervosa, anorexia nervosa or any other eating

disorder or issues of substance abuse (alcohol or drugs). Pregnancy, clinical depression or psychiatric care or a weight loss or gain of more than 5-kilograms in the previous 3-months were also exclusion factors. Participants were not to be actively following any diets such as Light and Easy, Jenny Craig, etc. Medications including immunosuppressants, amiodarone and/or perhexiline, or excessive consumption of vitamin E, vitamin C or vitamin D (>3000IU p/d) were criteria for exclusion. If participants had changed dosage of fish oil within the previous 3-months, this was recorded, as was alcohol consumption and change in weight prior to enrolment in the study.

4.5.4 Dietary Interventions

Following successful screening and enrolment into the trial, participants were randomised to either one of two dietary intervention arms by one researcher using computer-generated randomisation, stratified to sex and diabetes status. An Accredited Practicing Dietitian (APD) was allocated to the LFD group and another APD allocated to the MedDiet group to ensure that there was no potential cross-over of advice given to either diet group. Over the 12-week intervention period, each participant attended three face-to-face dietary consultation appointments (0-week, 6-week and 12-week) and received three phone-call follow up sessions (2-week, 4-week and 9-week) with their respective dietitian. Additional detail regarding the timing, content and data collection within these sessions is provided in **Chapter 2** (**Sections 2.6, 2.7, 2.8**). Both diets were delivered in an *ad-libitum* approach and weight-loss was not a main outcome of this study. Physical activity recommendations were not given to participants in either diet group, although if a participant had personal goals towards weight-loss or physical activity this was not discouraged instead it was recorded during their dietary consultation, as well as within a physical activity questionnaire (Active Australia Questionnaire) that was collected at each face-to-face appointment. Breakfast was provided to all participants in the form of a Jalna© yoghurt and Carmen's© muesli bar on the day of each face-to-face appointment after a fasting blood test was taken.

4.5.4.1 The Low-Fat Diet (LFD)

The LFD was based on dietary recommendations in line with the Australian Dietary Guidelines and the Heart Foundation advising dietary patterns.[101, 356] Specifically, advice was given to participants regarding portion sizes, low-fat options and cooking methods which was based on the Australian Guide to Healthy Eating.[356] The education and resources provided to participants in the LFD group were reflective of a dietary consultation in a typical outpatient diabetes clinic consult. Participants in the LFD group received a $20 Coles Supermarket gift voucher at the baseline, mid-intervention and end-intervention appointments to promote attendance and to assist them in purchasing some of the dietitian suggested food items.

4.5.4.2 The Mediterranean Diet (MedDiet)

The MedDiet intervention was based on a traditional MedDiet as previously published in the MEDINA Study protocol paper[277] and described in Keys et al. seven countries study.[523] The macronutrient composition of this MedDiet was reflective of a traditional Cretan Diet and comprised of approximately 44% fat (>50% monounsaturated fatty acids), 36% carbohydrates, 17-20% protein and up to 5% alcohol. A strong emphasis was placed on high consumption of plant-based foods including fruit and vegetables, whole grains, legumes and raw unsalted nuts, oily fish and the consumption of EVOO as the main culinary fat. Fermented dairy and white meat were recommended in moderate amounts, while red meat and sweets were recommended in small amounts. The dietitian advised changes taking into consideration each participants lifestyle, cultural and personal preferences and written resources designed to explain the MedDiet, it's components and principles were provided. Participants in the MedDiet group received a food hamper containing staple foods of a MedDiet including Cobram Estate © EVOO, nuts (Almond Board Australia), canned legumes, beans and fish (Simplot Australian Pty Ltd and HJ Heinz ©) at the baseline, mid-intervention and end-intervention appointments.

There was an expected cross-over with some food group recommendations given to participants in each dietary intervention groups, particularly regarding daily intake of fruit, vegetables and wholegrains, and limited intake of processed foods. However, the MedDiet also promoted key dietary components that the LFD did not, such as consumption of EVOO, legumes and nuts, oily fish and fermented dairy, and a lower consumption of red meat. The total energy intake of the MedDiet was envisaged to be higher than that of the LFD due to the composition of the of dietary fat recommendations. For example, monounsaturated fat would form >50% of total fat intake. Weight-gain was not expected in the MedDiet group based on previous studies that predict that the increased MUFA and omega-3 intake stimulate lipid oxidation.[524, 525]

4.5.5 Demographic Data

During the first face-to-face (0-week) appointment, a trained researcher (AR/EG) collected demographic information from each participant using a self-reported questionnaire (**Appendix 3.3**). Data collected included information regarding participant age, sex, marital and living status, level of education and current occupation, smoking status and ethnicity. Medication and supplement use were also recorded at baseline, and at each subsequent face-to-face appointment.

4.5.6 Anthropometric, Haemodynamic and Body Composition Measures

Using standard procedures in duplicate, a trained researcher collected participant weight, height, waist circumference (WC), hip circumference (HC) and neck circumference (NC), as well as systolic blood pressure (SBP) and diastolic blood pressure (DBP) at each of the face-to-face

appointments. Height (m) and weight (kg) were used to calculate BMI (kg/m^2), and waist and hip circumference were used to calculate waist-to-hip ratio. Reference ranges according to the World Health Organisation (WHO)[400] were followed. Body composition was also assessed at each face-to-face appointment using Seca© Bioelectrical Impedance Analysis (BIA) scales, complete with measures of fat mass (FM) in kilograms (kg) or percent (%), fat-free mass (FFM), fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle mass (SMM) and visceral fat (VF). Anthropometric and body composition measurements were collected from all participants at each face-to-face appointment; baseline (0-week), mid-intervention (6-week) and end intervention (12-week).

4.5.7 Primary Outcome Measures – Inflammatory Markers

The primary outcomes investigated in this study were inflammatory cytokines; hs-CRP, TNF-α, and IL-6, and adipokines; adiponectin, leptin and resistin. Blood samples were collected from participants using SST vacutainers and after centrifugation sera was separated and frozen at -80°C. Samples were collected, processed and stored by a trained MEDINA researcher at the Alfred Hospital (Gastroenterology Department) and once all participants had completed the intervention samples were transported to Deakin University for further analysis. Alongside collaborators from Deakin University, Melbourne, a trained MEDINA researcher (AR) assisted in the analysis of cytokine and adipokine markers using milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA) which simultaneously measured serum levels of cytokines (interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), cat num: HSTCMAG-28SK) and metabolic hormones (Adiponectin, Resistin and Leptin, cat num: HMHEMAG-34K) as previously described.[526] The assay was performed according to the manufacturer's instructions and all samples were run in duplicate. Inflammatory markers were measured at the baseline (0-week) and end intervention (12-week) timepoints.

4.5.8 Secondary Outcome Measures

Pathology blood samples were also collected at the Alfred Hospital and biomarker analysis of the following markers were carried out by Alfred Pathology Laboratory: insulin resistance (glucose, insulin), liver enzymes (total protein, albumin, globulin, bilirubin, ALT, AST, alkaline phosphatase (ALP), GGT and lipid studies (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides). These biomarkers were collected at each face-to-face appointment; baseline (0-week), mid-intervention (6-week), end-intervention (12-week). Homeostatic model assessment-Insulin Resistance (HOMA-IR) was calculated from fasting glucose and insulin levels (fasting glucose (mmol/L) x fasting insulin (mmol/L) / 22.5 = HOMA units) and was used as a surrogate marker to quantify disease severity in this cohort.

In this study, the magnetic resonance imaging (MRI) technique, proton magnetic resonance spectroscopy (^{1}H-MRS) was used to measure intrahepatic lipid (IHL) content. This is the gold-standard technique used to quantify hepatic steatosis.[11] All MRI studies were performed by a qualified radiographer who was blinded to participant study details. The studies were performed on an Avanto 1.5T system (Simmens©) machine at the Baker Heart and Diabetes Institute, The Alfred Centre, Melbourne.

Transient elastography (TE) Fibroscan™ was used to measure liver stiffness and fibrosis in this cohort. Following a minimum 2-hour fast each participant underwent ultrasound TE at 50Hz using a Fibroscan™ machine, performed and evaluated by a Hepatologist at the Alfred Hospital, Melbourne (Gastroenterology Department). Participants underwent ^{1}H-MRS and liver stiffness measure (LSM) assessment at baseline (0-week) and end intervention (12-week) appointments. Liver stiffness was reported in Kilo Pasqual's (kPa).

4.5.9 Dietary Assessment

Participants were required to complete 3-day food diaries prior to each face-to-face appointment (baseline (0-week), mid- (6-week) and end-intervention (12-week)) in order for habitual dietary intake and dietary compliance to be assessed. Participants were given instructions to detail and describe all food and beverage consumption to the best of their ability throughout two weekdays and one weekend day, and during each dietary consult the food diary was cross-checked by the dietitian to check for any missing detail or errors that could be corrected during the appointment. Portion sizes were also cross-checked, as well as branding of food products. Whilst the food diary was self-reported, the cross-checking by the dietitian was thought to provide additional accuracy in diet reporting. Although this was done, over- or under-reporting of dietary intake is expected as is known to be common in nutrition research. The 3-day food diaries were entered and analysed using FoodWorks8® using the AUSNUT 2013, AusBrands 2015 and AusFoods 2015 databases. Macronutrient, micronutrient and food group analysis was exported from FoodWorks9™ and further analysis was conducted in SPSS® statistical package version 25 (IBM Corp, Released 2017).

4.5.10 PREDIMED Score

Adherence to a Mediterranean Diet was assessed using a 14-point checklist which was developed and validated by MedDiet researchers in Spain, who were investigating the effects of a MedDiet in cardiovascular disease.[360] The checklist is comprised of key components (foods and beverages) characteristic of a traditional Mediterranean Diet, each item is worth one point for inclusion or zero for exclusion from diet. Therefore, greater adherence will score higher in points on the 14-point checklist and less adherence will score closer to '0'. An example of this checklist is presented in **Appendix 6.2**. The checklist was completed by each participant in the MedDiet group prior to each

face-to-face appointment on the trial and the checklist as reviewed by the consulting dietitian during their dietary consult to ensure participants understood the checklist and to minimise errors or inconsistencies. For the LFD group, MedDiet adherence scores were calculated retrospectively so that comparisons could be made between groups for MedDiet adherence.

A separate 9-item PREDIMED checklist was used as a crude measure for adherence to a LFD in the LFD group. This checklist was also developed by the aforementioned PREDIMED Study and contained key components of the LFD and questions regarding low-fat cooking methods, for which participants scored one point for adherence and zero for non-adherence. The greater the score (up to 9), implied the greater adherence to a LFD. An example of this checklist is presented in **Appendix 6.3**. Each participant in the LFD group completed this checklist prior to each face-to-face appointment on the trial and the checklist as reviewed by the consulting dietitian during their dietary consult to ensure participants understood the checklist and to minimise errors or inconsistencies.

4.5.11 Statistical Analysis

Prior to performing any statistical analyses, data was checked for obvious data entry errors and outliers. If an error was found or data was deemed uncertain, original data was checked, and where possible the error was corrected. Outliers were defined as observations above or below ± 2.24 standard deviation (SD) unit of the mean.[527] There were no outliers excluded for this analysis as all were deemed correct and true values. Data that was available for participants at each timepoint was utilised in all analyses. Completers of the intervention (n=39) were included in post-intervention analysis, where data did not exist for a participant (drop-out or missed appointment) they were not included.

All statistical analysis was performed by one independent researcher (AR) using the Statistical Package for the Social Sciences (SPSS), version 25 (IBM Corp, Released 2017). Normality of variables was assessed using Kolmogorov-Smirnov statistic and normality was indicated by a non-significant result ($p > 0.05$).

Throughout this chapter, irrespective of normality, descriptive statistics are reported as mean ± standard deviation (SD) and categorical variables presented as frequencies (n) and percentages (%). This was done so that meaningful comparisons could be made between outcomes at each timepoint, however data was statistically analysed according to normality of each variable.

4.5.11.1 Section One: The effects of a dietary intervention in patients with NAFLD

For between groups analyses of the LFD and MedDiet groups, independent samples t-tests were used for parametric data and Mann-Whitney U Tests were used for non-parametric data ($p < 0.05$) for the analyses of the differences in outcome variables between the two groups. For within dietary intervention group analyses between the baseline and end-intervention timepoints, paired samples

t-tests were used for parametric data and Wilcoxon Signed Rank Tests were used for non-parametric data ($p < 0.05$). In order to assess the differences between the baseline (0-week), mid-intervention (6-week) and end-intervention (12-week) timepoints, one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative. Post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test) as appropriate. The p-value for Bonferroni corrected tests was adjusted appropriately for testing across three timepoints ($p < 0.017$).

Partial correlation was used to determine the strength and direction of the relationship between each inflammatory marker (cytokines and adipokines) with liver outcomes, blood biomarkers, anthropometry and body composition measures and nutrient and food group intake within each diet group while controlling for change in weight between baseline and end intervention. This was done to ensure that any change in weight – particularly weight loss – between the baseline and end-intervention timepoints which may influence the variables of interest, was removed. Eliminating weight-loss as a confounding factor allows for a clear and more accurate indication of the relationship between variables. R-values were classified as weak (0.10 to 0.29), moderate (0.30 to 0.49) or strong (0.50 to 1.00) correlations.[528]

Total PREDIMED Scores for dietary adherence to a MedDiet for the MedDiet and LFD groups are presented as mean ± SD, and between group differences calculated using independent samples t-tests at the baseline and end-intervention timepoints. Change in the PREDIMED MedDiet scores from baseline to end-intervention within each dietary intervention group were calculated using paired samples t-tests. Compliance to each dietary component on the checklist (14-items) were also presented as mean ± SD and change in adherence between the baseline and end-intervention timepoints within each dietary intervention group was calculated using paired samples t-tests.

Adherence to a LFD was also assessed using a separate PREDIMED checklist in the LFD group only, and data was presented as mean ± SD and change in adherence between the baseline and end-intervention timepoints within the LFD intervention group was calculated using paired samples t-tests.

To assess the impact of adherence to a MedDiet and improved diet quality at the end of intervention, PREDIMED scores for the overall cohort were divided into two categories labelled “higher adherence” and “lower adherence” derived from above and below mean values for the end-intervention PREDIMED score. Mean ± SD values of inflammatory markers, liver outcomes, HOMA-IR and key anthropometric and body composition variables were assessed between levels of adherence. All measurements were from the end intervention timepoint and included the entire (pooled) cohort. Significance between the two groups was identified using independent samples t-tests for parametric data and Mann-Whitney U Tests for non-parametric data.

Partial correlation was used to determine the strength and direction of the relationship between each inflammatory marker (cytokines and adipokines) with nutrients and food groups known to be associated with the MedDiet, while controlling for the baseline value of the inflammatory marker. Partial correlations were also conducted to determine the linear relationship between inflammatory markers with liver outcomes and blood biomarkers, anthropometry, body composition and nutrient and food group intake, after controlling for weight change between baseline and end intervention.

Multiple linear regression analyses were used to generate prediction equations for significantly ($p < 0.05$) correlated variables with each inflammatory marker at the post-intervention time point. Firstly, models were conducted using key nutrients and food groups of the MedDiet input as independent variables and each inflammatory marker input as the dependent variable. Separate models were conducted with anthropometric, body composition, biochemical and all dietary intake variables as the independent variable and each inflammatory marker as the dependent variable. For each regression model, non-parametric variables; hs-CRP, IL-6 and leptin were included as log-transformed variables in order to conform to normality. In each regression model, the inflammatory marker at the post-intervention timepoint was input as the dependent variable and the baseline value of the inflammatory marker was adjusted for in the model. Age, sex, diet group, diabetes status and body weight (kg) at baseline were controlled for in each regression model. Randomisation to either dietary intervention group was controlled for as this analysis intended to find predictors of inflammatory markers regardless of the influence of dietary intervention. Diabetes status was controlled for due to the significant difference in insulin and HOMA-IR between participants with and without diabetes. Body weight (kg) was controlled for to ensure that weight change between timepoints did not influence the predictive ability of models. Age and sex were considered potential confounders and were controlled for in each model. Before interpreting the results, a number of assumptions were tested, and checks were performed. Assessment of the normal probability plot of standardised residuals and the scatterplot of standardised residuals against predicted values indicated that the assumptions of normality, linearity and homoscedasticity were met. If two variables were similar in nature or one variable encompassed another (for example, PUFA (%E) and linoleic acid), only the variable with the strongest correlation was included in the regression model. Potential violations of the assumption of multicollinearity was assessed by collinearity diagnostic values for Tolerance and variance inflation factor (VIF). Standardised beta (β) regression coefficients, squared multiple correlation (R^2) or squared-change correlation (ΔR^2), regression and residual degrees of freedom (df1 and df2, respectively) and significance level (P-value) were reported for each model.

4.5.11.2 Section Two:

Assessing the impact of a dietary intervention and overall improved diet quality in patients diagnosed with NAFLD and T2DM – Subgroup Analysis

Statistical analysis was undertaken to assess the impact of dietary intervention in individuals with diagnosed T2DM in comparison to individuals without diagnosed T2DM. This was done due to the number of participants with diagnosed T2DM in the MedDiet (n=7) and LFD (n=11) groups at baseline which may have an effect on the overall intervention changes observed. There were a much greater number of females diagnosed with diabetes compared to males (1M vs 17F). The published MEDINA Study Protocol [277] was initially powered to detect a between-group difference in HOMA-IR and this study was powered to n=94 (47 participants per arm). Upon enrolment into the trial, participants underwent stratified randomisation based on sex and diabetes status which was set-up to account for a full cohort of 94 participants. Thus, the low number of participants who were actually recruited to trial (n=42) is thought to have affected this randomisation process and contributed to the sex and diabetes differences between groups (albeit, not significant). It is anticipated that the uneven spread of sex and individuals with diabetes between groups will resolve upon recruitment of the full cohort.

In order to assess the changes in outcomes following MedDiet and LFD interventions in the diabetes cohort, statistical analyses were conducted for the key outcomes: anthropometry and body composition, biochemistry, liver and inflammatory markers. Data were first split by diabetes status (T2DM vs non-T2DM) and intervention group (MedDiet vs LFD), and key outcomes were reported as mean ± SD for baseline and end-intervention timepoints. Within the group diagnosed with T2DM and the group without T2DM, between groups analyses of the LFD and MedDiet groups was assessed using independent samples t-tests for parametric data and Mann-Whitney U Tests for non-parametric data. To analyse change in each key outcome (anthropometry and body composition, biochemistry, liver and inflammatory markers) in the pooled cohort between the baseline and end-intervention timepoints within the T2DM and non-T2DM groups, paired samples t-tests were used for parametric data and Wilcoxon Signed Rank Tests were used for non-parametric data.

4.5.12 Power Calculation

The power calculation for the overarching MEDINA Trial can be found in Chapter 2 (**Section 2.11**). The sample of participants recruited for this study was based on IHL summary data in Table 2 of Ryan et al. (2013) with the assumption of a 25% change of IHL in the MedDiet group and 5% in the LFD group, resulting in difference of 20% in change of IHL.[499] The required sample size for each group was 17 (with a power of 80%, $\alpha<0.05$). Adjusting for a potential 20% dropout, the required sample size is 17/0.8 = 21 participants per group. Therefore, the total sample size of 42 participants without dropouts was achieved.

A sample size calculation powered to see a change in each inflammatory cytokine and adipokine was conducted prior to analysis of outcomes. The candidates systematic review [256] provided insight into the effects of various dietary interventions on inflammatory markers and using results from these studies, *a priori* power analysis was based on the statistical test for 'Means: Difference between two independent means (two groups)'. Calculation of the sample size required to detect a significant difference between groups for hs-CRP, TNF-α, IL-6, adiponectin, resistin and leptin was performed using statistical software program G*Power 3.0.10. The calculation included the effect size, power (1-β err prob) of 80% and $\alpha<0.05$. The estimated sample size prediction required between 120 – 350 participants, depending on the sensitivity of each inflammatory marker. As such, this study is a pilot and feasibility study for the purpose of these analyses. These calculations are available in **Appendix 9**.

4.5.13 Ethics

Human research ethics committee approval was obtained for all recruitment sites, including Alfred Health, Eastern Health, The Royal Melbourne Hospital and St Vincent's Hospital. The La Trobe University human research ethics committee also provided approval for the trial. Approval certificates are available in **Appendix 8**.

4.6 Results

4.6.1 Population Characteristics

A total of 42 participants were recruited and randomised to either the LFD (n=23) or MedDiet (n=19) intervention group of the MEDINA study. From baseline to end-intervention, a total of three participants withdrew from the study, representing an attrition rate of 7%. Two participants from the LFD withdrew due to family and medical reasons and one participant from the MedDiet withdrew due to personal circumstances. Data was collected for all 42 participants at the baseline timepoint; at the mid-intervention timepoint data for 36 participants and 39 participants completed the study. A flow diagram of study participation is shown in **Figure 4.1.**

Of the 23 participants in the LFD group 14 (61%) were female and of the 19 participants in the MedDiet group, 11 (58%) were female. There was no significant difference between the mean age of participants in each dietary intervention group at baseline (LFD 52.1 ± 13.6 years vs. MedDiet 52.6 ± 11.7 years, p =0.90, respectively).

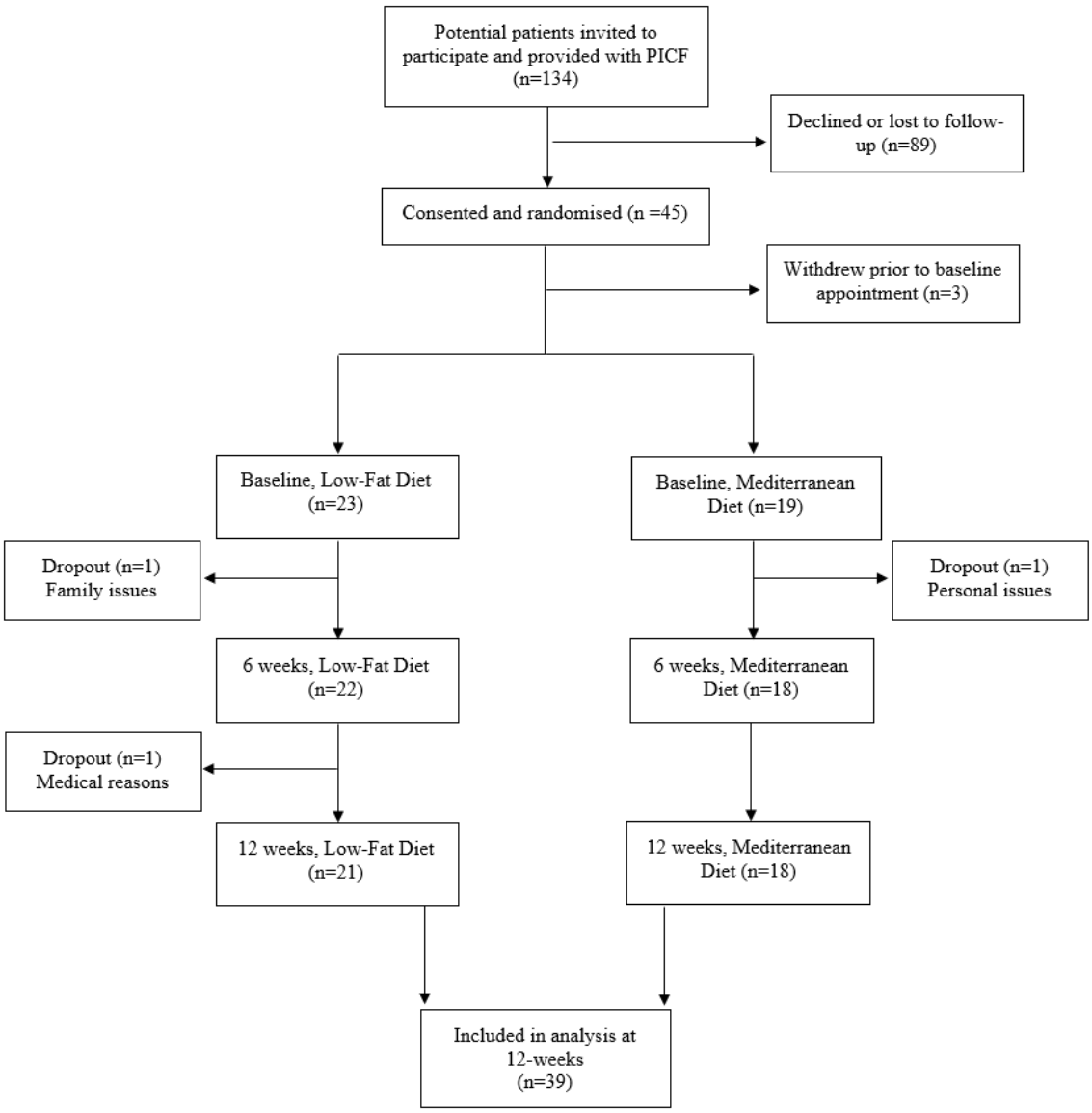


Figure 4.1. Study patient flow chart for the MEDINA Study. Summary of participant recruitment and number of participants whose data was collected at each timepoints.

4.7 Section One – The effects of a dietary intervention in patients with NAFLD

4.7.1 Differences between the MedDiet and LFD intervention groups for Inflammatory Markers at 12-weeks – end intervention and absolute change data

No significant differences in inflammatory markers were noted between the two dietary intervention groups at the post intervention time point **(Table 4.1)**. Of the inflammatory markers assessed, the absolute change in leptin was significant between the diet groups. Participants allocated to the LFD group reduced levels of leptin, compared to no change in the MedDiet group; -1.20 ± 3.9% vs. 0.64 ± 3.5%, p=0.038.

4.7.2 Effects of the Dietary Intervention on Inflammatory Markers– within diet group effects

Table 4.1 presents the mean values for inflammatory markers, IHL and LSM at baseline and end-intervention in the overall cohort and LFD and MedDiet groups, and mean change in each variable from baseline to end-intervention. At baseline, n=3 participants had a measurement of >10 mg/L for hs-CRP indicating an acute inflammatory state.[156] The hs-CRP measurement value for these participants were excluded from all data analysis. At the end-intervention timepoint, n=6 participants had hs-CRP measurement >10.0 mg/L and their hs-CRP values were also excluded from all data analysis. There was a no change in hs-CRP following both LFD and MedDiet (3.8 ± 2.7 mg/L to 3.5 ± 2.6 mg/L, p =0.332, and 2.6 ± 2.3 mg/L to 2.2 ± 1.9 mg/L, p =0.814, respectively). A decrease in TNF-α was observed in the LFD group, although not significant (6.1 ± 6.4 pg/mL to 4.5 ± 2.0 pg/mL, p =0.227). The MedDiet group experienced a small, non-significant increase in TNF-α (3.8 ± 2.0 pg/mL to 4.1 ± 1.9 pg/mL, p =0.507). There were no significant changes observed for IL-6 in either diet group.

Both dietary intervention groups experienced improved levels of circulating serum adiponectin, however this change was only significant in the MedDiet group (13.7 ± 9.2 µg/mL to 17.0 ± 12.5 µg/mL, p =0.016). Serum resistin and leptin decreased in the LFD group, though changes were small and non-significant, and did not change in the MedDiet group. There was a significant difference in the mean change between the LFD and MedDiet groups, wherein leptin levels decreased significantly more with the LFD compared to the MedDiet group (-1.20 ± 3.85 ng/mL vs 0.64 ± 3.51 ng/mL, p =0.038).

4.7.3 Differences between the MedDiet and LFD intervention groups for Liver Outcomes and Biochemistry at 12-weeks – end intervention and absolute change data

Table 4.1 and **Table 4.2** present the end-intervention and absolute change data for liver outcomes and biochemistry between the MedDiet and LFD groups No significant differences between the MedDiet and LFD groups were observed for liver outcomes and biochemistry at the post intervention time point. The change in fasting insulin and HOMA-IR decreased by a greater percentage in the LFD group compared to the MedDiet group (-3.91 ± 5.9% vs -0.06 ± 6.4%, p =0.016, and -1.16 ± 2.2% vs -0.38 ± 2.7%, p =0.022, respectively). Circulating levels of liver enzymes ALT, AST and GGT decreased in the LFD group and increased in the MedDiet group, the change between groups was significant for each (ALT -17.71 ± 31.3% vs 9.94 ± 34.9%, p =0.022, AST -9.52 ± 20.5% vs 8.28 ± 22%, p =0.012, and GGT -38.52 ±86.7% vs 11.22 ± 45.2%, p =0.037, respectively).

4.7.4 Effects of the Dietary Intervention on Liver Outcomes and Biochemistry

At baseline, IHL content was significantly higher in participants randomised to the MedDiet than those in the LFD (13.7 ± 7.8% vs 9.2 ± 10.6%, p =0.049)(**Table 4.1**). There was no significant change in IHL content between baseline and end intervention in the MedDiet or LFD. Conversely, LSM was non-significantly higher in the LFD group at baseline; the LFD group experienced reductions in LSM from pre- to post-intervention (11.8 ± 14.3 kPa to 10.8 ± 10.2 kPa, p =0.13) and remained the same for the MedDiet group between timepoints (7.8 ± 4.0 kPa to 7.6 ± 5.2 kPa, p =0.32).

Table 4.2 displays the mean values for HOMA-IR and biochemical markers at baseline, mid- and end-intervention in the overall cohort and LFD and MedDiet groups, and the mean change from baseline to end-intervention for each variable. There were significant reductions in fasting insulin and HOMA-IR in the LFD group (20.0 ± 12.4 mIU/L to 16.4 ± 11.3 mIU/L, p =0.031, and 6.5 ± 5.6 to 5.5 ± 5.5, p =0.047, respectively). Although not significant, noteworthy clinical reductions were observed for fasting insulin and HOMA-IR in the MedDiet group (16.4 ± 8.9 mIU/L to 15.5 ± 8.5 mIU/L, p =0.374, and 4.4 ± 3.2 to 3.9 ± 2.3, p =0.459, respectively). Liver enzymes ALT, AST, and GGT were significantly reduced following the LFD intervention (61.5 ± 37.0 U/L to 46.9 ± 20.8 U/L, p =0.05, 41.8 ± 21.6 U/L to 34.1 ± 15.4 U/L, p =0.004, and 126.6 ± 128.8 U/L to 95.2 ± 72.9 U/L, p =0.028, respectively). There were moderate, non-significant increases in AST, ALT, and GGT following the MedDiet intervention (54.1 ± 25.2 U/L to 64.7 ± 39.5 U/L, p =0.717, 31.8 ± 12.5 U/L to 39.7 ± 27.4 U/L, p =0.362, 90.0 ± 74.6 U/L to 105.1 ± 91.2 U/L, p =0.298, respectively). No significant change in ALP was seen in either diet group (p >0.05). Fasting glucose was significantly higher in the LFD group compared to the MedDiet group at baseline (6.7 ± 1.9 mmol/L vs 5.8 ± 1.6 mmol/L, p =0.028), though remained unchanged in both groups from baseline to end-intervention (p >0.05).

Table 4.1. Inflammatory cytokine and adipokine markers and liver outcomes at baseline and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P[a]	P[b]		
	n	mean	SD	n	mean	SD	n	mean	SD		Total	LFD	MedDiet
hs-CRP (mg/L)													
Baseline	38	3.27	2.56	21	3.84	2.69	17	2.56	2.27	0.121	0.596	0.332	0.814
End-Intervention	33	2.93	2.40	19	3.47	2.64	14	2.19	1.87	0.186			
Change		-0.07	1.47		-0.32	1.25		-0.25	1.69	0.419			
TNF-α (pg/mL)													
Baseline	41	5.01	4.93	22	6.05	6.35	19	3.81	2.04	0.071	0.310	0.227	0.507
End-Intervention	39	4.32	1.97	21	4.52	2.04	18	4.10	1.91	0.53			
Change		-0.70	4.19		-1.46	5.66		0.15	1.01	0.196			
IL-6 (pg/mL)													
Baseline	41	11.17	18.04	22	7.89	9.55	19	14.97	24.25	0.824	0.078	0.681	0.076
End-Intervention	39	13.78	20.22	21	11.39	14.31	18	16.58	25.64	0.770			
Change		0.84	3.69		0.80	4.79		0.88	2.01	0.276			
Adiponectin (µg/mL)													
Baseline	41	15.60	12.24	22	17.25	14.37	19	13.68	9.22	0.657	0.088	0.823	0.016*
End-Intervention	39	18.36	17.47	21	19.53	21.04	18	17.01	12.55	0.856			
Change		2.48	11.32		1.83	14.78		3.21	5.80	0.303			
Resistin (ng/mL)													
Baseline	41	39.68	18.73	22	41.61	22.64	19	37.45	13.12	0.647	0.274	0.500	1.000
End-Intervention	39	39.43	18.30	21	39.00	20.90	18	39.94	15.32	0.777			
Change		0.09	11.43		-1.44	12.81		1.78	9.76	0.965			
Leptin (ng/mL)													
Baseline	41	16.27	12.15	22	18.65	13.60	19	13.50	9.86	0.174	0.547	0.100	0.472
End-Intervention	39	15.29	11.81	21	16.70	13.13	18	13.65	10.16	0.477			
Change		-0.33	3.76		-1.20	3.85		0.64	3.51	0.038*			

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b		
	n	mean	SD	n	mean	SD	n	mean	SD		Total	LFD	MedDiet
Liver Outcomes													
IHL (%)													
Baseline	33	11.39	9.51	17	9.20	10.65	16	13.70	7.80	0.049*	0.065	0.35	0.08
End-Intervention	31	10.45	10.39	16	8.94	12.43	15	12.06	7.79	0.093			
Change		-0.60	3.80		-0.18	4.81	15	-1.06	2.39	0.830			
LSM (kPa)													
Baseline	42	9.99	11.02	23	11.82	14.33	19	7.76	4.03	0.677	0.084	0.126	0.32
End-Intervention	37	9.26	8.22	19	10.80	10.23	18	7.63	5.19	0.62			
Change		-1.31	5.26		-2.46	6.45		-0.10	3.40	0.48			

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IHL, Intrahepatic Lipid; LSM, liver stiffness measure. All data presented as mean ± SD. * indicates significance ($p < 0.05$). *Change* represents absolute change in variable from baseline to end-intervention. P^a p-values for comparing differences between participants in each diet group at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data.

Table 4.2. HOMA-IR and biochemical measures at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
Glucose (mmol/L)														
Baseline	42	6.33	1.86	23	6.75	1.98	19	5.82	1.62	0.028*				
Mid-Intervention	34	6.26	1.73	17	6.78	1.93	17	5.73	1.35	0.024*	0.893	0.906	0.819	0.480
End-Intervention	39	6.27	1.96	21	6.75	2.38	18	5.71	1.14	0.14		0.690		0.704
Change		-0.08	1.37		-0.01	1.75		-0.17	0.75	0.835				
Insulin (mIU/L)														
Baseline	42	18.37	10.99	23	20.01	12.38	19	16.39	8.95	0.337	0.031*		0.374	

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
Mid-Intervention	34	16.72	10.52	17	20.57	13.27	17	12.88	4.56	0.049*		0.670		0.215
End-Intervention	39	15.98	9.99	21	16.41	11.28	18	15.47	8.54	0.686		0.065		0.309
Change		-2.14	6.38		-3.91	5.94		-0.06	6.41	0.016*				
HOMA-IR														
Baseline	42	5.55	4.72	23	6.49	5.56	19	4.41	3.24	0.129				
Mid-Intervention	34	4.98	4.24	17	6.59	5.34	17	3.37	1.76	0.014*	0.047*	0.687	0.459	0.255
End-Intervention	39	4.75	4.38	21	5.48	5.53	18	3.90	2.33	0.686		0.059		0.670
Change		-0.80	2.43		-1.16	2.19		-0.38	2.69	0.022*				
ALT (U/L)														
Baseline	42	58.14	32.07	23	61.48	37.01	19	54.11	25.24	0.752				
Mid-Intervention	34	50.12	32.56	17	45.53	28.49	17	54.71	36.47	0.413	0.05*	0.016**	0.717	0.679
End-Intervention	39	55.13*	31.73	21	46.90	20.85	18	64.72	39.47	0.269		0.552		0.368
Change		-4.95	35.47		-17.71	31.33		9.94	34.94	0.022*				
AST (U/L)														
Baseline	42	37.29	18.58	23	41.83	21.61	19	31.79	12.55	0.172				
Mid-Intervention	34	34.85	27.27	17	34.71	20.58	17	35.00	33.32	0.518	0.004*	0.050	0.362	0.346
End-Intervention	39	36.67*	21.64	21	34.10	15.38	18	39.67	27.41	0.856		0.139		0.522
Change		-1.31	22.76		-9.52	20.47		8.28	22.01	0.012*				
GGT (U/L)														
Baseline	42	110.10	108.11	23	126.65	128.81	19	90.05	74.63	0.419				
Mid-Intervention	34	82.97	78.19	17	90.41	98.80	17	75.53	52.26	1.000	0.028*	0.050	0.298	0.195
End-Intervention	39	99.74*	80.92	21	95.19	72.93	18	105.06	91.23	0.856		0.569		0.379
Change		-15.56	74.21		-38.52	86.77		11.22	45.18	0.037*				
ALP (U/L)														
Baseline	42	92.45	30.57	23	93.26	33.34	19	91.47	27.72	0.96	0.602		0.869	
Mid-Intervention	34	92.94	27.61	17	95.06	30.14	17	90.82	25.58	0.812		0.585		0.812

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
End-Intervention	39	95.41	27.65	21	94.19	24.86	18	96.83	31.28	0.967		0.683		0.660
Change		1.31	20.91		-0.29	24.08		3.17	17.00	0.728				
Bilirubin (µmol/L)														
Baseline	42	14.24	8.80	23	12.30	6.74	19	16.58	10.50	0.149				
Mid-Intervention	34	15.12	8.51	17	13.24	6.73	17	17.00	9.83	0.231	0.074	0.954	0.638	0.776
End-Intervention	39	14.77	9.79	21	13.10	8.11	18	16.72	11.36	0.321		0.394		0.696
Change		0.44	4.41		1.05	4.77		-0.28	3.95	0.460				
Total Protein (g/L)														
Baseline	42	76.40	5.64	23	77.57	6.14	19	75.00	4.74	0.144				
Mid-Intervention	34	74.88	5.35	17	75.88	5.42	17	73.88	5.25	0.283	0.097	0.089	0.777	0.529
End-Intervention	39	75.44	4.69	21	76.48	4.91	18	74.22	4.24	0.136		0.734		0.910
Change		-0.74	4.08		-1.24	4.27		-0.17	3.88	0.420				
Albumin (g/L)														
Baseline	42	39.69	3.29	23	39.13	3.17	19	40.37	3.39	0.229				
Mid-Intervention	34	39.18	2.50	17	38.53	2.32	17	39.82	2.58	0.134	0.046*	0.230	0.411	0.490
End-Intervention	39	38.77*	2.25	21	38.10	2.41	18	39.56	1.82	0.042*		0.955		0.346
Change		-0.74	2.20		-1.00	2.10		-0.44	2.33	0.438				
Globulin (g/L)														
Baseline	42	36.74	5.73	23	38.48	6.03	19	34.63	4.66	0.159				
Mid-Intervention	34	35.88	5.31	17	37.35	5.70	17	34.41	4.60	0.339	0.171	0.019*	0.545	0.793
End-Intervention	39	36.69	5.37	21	38.43	5.43	18	34.67	4.67	0.148		0.035*		0.776
Change		0.00	3.06		-0.24	3.00		0.28	3.20	0.749				
Cholesterol (mmol/L)														
Baseline	42	4.96	1.55	23	4.87	1.50	19	5.08	1.63	0.552				
Mid-Intervention	34	4.93	1.38	17	4.77	1.42	17	5.09	1.35	0.433	0.175	0.494	0.664	0.698
End-Intervention	39	4.91	1.48	21	4.74	1.43	18	5.11	1.56	0.568		0.592		0.608

Outcome	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
Change		-0.10	0.70		-0.22	0.60		0.04	0.79	0.426				
HDL (mmol/L)														
Baseline	42	1.19	0.25	23	1.21	0.27	19	1.16	0.22	0.628				
Mid-Intervention	34	1.17	0.28	17	1.14	0.24	17	1.21	0.31	0.683	0.368	0.156	0.480	0.164
End-Intervention	39	1.19	0.27	21	1.19	0.28	18	1.19	0.27	0.878		0.132		0.319
Change		0.00	0.19		-0.03	0.23		0.03	0.13	0.53				
LDL (mmol/L)														
Baseline	42	3.03	1.30	23	2.86	1.33	19	3.23	1.28	0.37				
Mid-Intervention	34	2.95	1.12	17	2.82	1.15	17	3.07	1.10	0.527	0.892	0.871	0.493	0.209
End-Intervention	39	2.97	1.30	21	2.81	1.31	18	3.15	1.30	0.422		0.742		0.496
Change		-0.10	0.51		-0.05	0.57		-0.08	0.45	0.702				
Triglycerides (mmol/L)														
Baseline	42	1.78	0.87	23	1.78	0.88	19	1.77	0.88	0.99				
Mid-Intervention	34	1.79	0.91	17	1.80	0.81	17	1.78	1.02	0.474	0.586	0.842	0.159	0.468
End-Intervention	39	1.67	0.73	21	1.65	0.70	18	1.71	0.77	0.100		0.381		0.598
Change		-0.10	0.57		-0.14	0.71		-0.06	0.37	0.728				

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HOMA-IR, homeostatic model assessment-insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein. All data presented as mean ± SD. * indicates significance ($p < 0.05$), ** indicates significance ($p < 0.01$). *Change* represents absolute change in variable from baseline to end-intervention. P^a p-values for comparing differences between participants in each diet group at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values compares differences between baseline, mid-intervention and end-intervention timepoints in the control (LFD) group and P^d compares differences between baseline, mid-intervention and end-intervention timepoints in the intervention (MedDiet) group; one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative; post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test)($p<0.017$).

4.7.5 Effects of Dietary Intervention on Anthropometry, Body Composition and Haemodynamic measures

Table 4.3 presents the mean values for anthropometry, body composition and haemodynamic measures at baseline, mid- and end-intervention in the overall cohort and LFD and MedDiet groups, and the mean change from baseline to end-intervention of each variable.

Interestingly, the LFD group experienced a non-significant increase in body weight (kg) from baseline to mid-intervention (88.9 ± 22.7 kg to 93.1 ± 25.6 kg, p =1.000), followed by a noteworthy but non-significant decrease of 7.3 kg from mid- to end-intervention (93.1 ± 25.6 kg to 85.8 ± 18.1 kg, p =0.210). From baseline to end intervention, mean weight change in the LFD group was -4.02 ± 2.29 kg (p =0.382). Conversely, the MedDiet group experienced no significant change in body weight from baseline to mid-intervention (87.7 ± 21.1 kg to 89.1 ± 24.5 kg, p =0.873), and this remained unchanged from the mid- to end-intervention timepoint (89.1 ± 24.5 kg to 89.3 ± 22.8 kg, p =0.964). From baseline to end intervention, mean weight change in the MedDiet group was 0.90 ± 1.79 kg. In the LFD group mean values of BMI (kg/m^2) and WC (cm) decreased notably, albeit non-significantly, in line with the weight loss observed in the group. No significant changes were observed for BMI (kg/m^2) and WC (cm) in the MedDiet group. No significant changes were observed for neck or hip circumference in either diet group (data not shown).

Although it was not a significant finding, fat mass (%) decreased in the LFD group (40.8 ± 7.8% to 38.9 ± 7.9%, p =0.189, respectively) which is reflective of the weight loss observed. VF (L) was significantly greater in the LFD group than the MedDiet group at baseline (4.4 ± 2.1L vs 3.2 ± 1.5L, p =0.017) and although both diet groups experienced a significant reduction in VF content from baseline to end-intervention, the reduction was significantly greater in the LFD group compared to the MedDiet group (4.4 ± 2.1L to 1.5 ± 0.6L, p =0.0005, vs 3.2 ± 1.5L to 1.8 ± 1.4L, p =0.0005). The change in VF was similar to that of body weight; VF increased slightly and non-significantly from baseline to mid-intervention, then decreased significantly from mid- to end-intervention in both the LFD (4.4 ± 2.1L to 5.1 ± 2.9L, p =0.255, and 5.1 ± 2.9L to 1.5 ± 0.6 L, p <0.001, respectively) and MedDiet groups (3.2 ± 1.5L to 4.1 ± 2.5L, p =0.289, and 4.1 ± 2.5L to 1.8 ± 1.4L, p <0.001, respectively). Visceral fat decreased in participants allocated to both the LFD and MedDiet groups, though the change was greater in the LFD group; -3.05 ± 1.9% vs -1.4 ± 2.4%, p =0.014. There were no significant findings within or between groups or across the time points for other measures of body composition FM (kg), FFM (kg), FMI (kg/m^2), FFMI (kg/m^2) and SMM (kg) (p> 0.05)(data available in **Appendtix 10; Supplementary Table 3**).

Haemodynamic measures of SBP significantly decreased in the LFD group from baseline to end-intervention (127.4 ± 19.2 mmHg to 118.6 ± 10.8 mmHg, p =0.038) and diastolic blood pressure (DBP) and heart rate decreased non-significantly (83.3 ± 9.8 mmHg to 80.3 ± 8.6 mmHg, p =0.059, and 77.5 ± 14.4 bpm to 75.0 ± 14.5 bpm, p =0.306, respectively). Haemodynamic measures SBP,

DBP and heart rate fluctuated non-significantly in the MedDiet group. Of note, at baseline, heart rate was significantly higher in the LFD group compared to the MedDiet group (77.5 ± 14.4 bpm vs 69.5 ± 9.4 bpm, p =0.042).

Table 4.3. Anthropometry, body composition and haemodynamic measures at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups.

Characteristics	Total			Control (LFD)			Intervention (MedDiet)			P[a]	P[b]			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P[c]	MedDiet	P[d]
Anthropometry														
Weight (kg)														
Baseline	42	88.86	22.74	23	89.80	24.44	19	87.72	21.11	0.773	0.382		0.623	
Mid-Intervention	36	91.07	24.79	18	93.09	25.58	18	89.06	24.54	0.633		0.467		0.873
End-Intervention	39	87.40	20.22	21	85.78	18.14	18	89.28	22.79	0.597		0.210		0.964
Change		-0.69	9.28		-4.02	10.50		0.90	7.60	0.329				
BMI (kg/m^2)														
Baseline	42	32.22	6.27	23	32.72	6.95	19	31.61	5.45	0.574	0.574		0.956	
Mid-Intervention	36	32.26	6.60	18	32.88	7.12	18	31.65	6.17	0.581		0.310		0.982
End-Intervention	39	31.55	5.15	21	31.29	4.91	18	31.85	5.54	0.74		0.301		0.840
Change		-0.31	2.96		-0.77	3.57		0.22	2.01	0.306				
WC (cm)														
Baseline	42	107.10	17.03	23	108.74	18.89	19	105.11	14.71	0.640	0.137		0.946	
Mid-Intervention	36	107.60	18.46	18	108.94	20.62	18	106.26	16.51	0.767		0.316		0.845
End-Intervention	39	104.99	13.77	21	103.92	12.67	18	106.24	15.24	0.090		0.501		0.744
Change		-2.10	8.74		-4.82	9.17		1.13	8.07	0.223				
WHR														
Baseline	42	0.97	0.06	23	0.98	0.06	19	0.96	0.06	0.327	0.318		0.412	
Mid-Intervention	36	0.98	0.05	18	0.97	0.06	18	0.98	0.03	0.502		0.313		0.445
End-Intervention	39	0.96	0.06	21	0.97	0.05	18	0.96	0.06	0.473		0.577		0.204
Change		-0.01	0.04		-0.01	0.05		-0.01	0.04	0.931				
Blood pressure (mmHg)														
Systolic														
Baseline	42	126.52	16.26	23	127.43	19.23	19	125.42	12.15	0.695	0.038*		0.715	

Characteristics	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
Mid-Intervention	35	120.60	14.49	17	116.35	14.28	18	124.61	13.88	0.092		0.016*		0.322
End-Intervention	37	120.76	12.14	20	118.58	10.82	17	123.32	13.41	0.241		0.499		0.676
Change		-5.70	14.58		-8.85	14.90		-2.68	14.35	0.443				
Diastolic														
Baseline	42	83.12	8.60	23	83.35	9.84	19	82.84	7.07	0.852	0.059		0.991	
Mid-Intervention	35	81.81	9.34	17	79.29	9.93	18	84.19	8.33	0.123		0.039*		0.905
End-Intervention	36	81.89	8.46	19	80.26	8.65	17	83.71	8.11	0.228		0.121		0.736
Change		-1.92	10.92		-3.79	11.43		0.18	10.25	0.283				
Heart Rate (bpm)														
Baseline	40	73.69	12.77	21	77.50	14.38	19	69.47	9.37	0.042*	0.306		0.907	
Mid-Intervention	35	72.83	10.93	17	74.88	11.70	18	70.89	10.09	0.287		0.445		0.992
End-Intervention	37	73.09	14.07	20	75.00	14.55	17	70.85	13.58	0.379		0.495		0.805
Change		-0.71	13.97		-2.61	14.90		1.41	12.98	0.397				
Body Composition														
Fat mass (%)														
Baseline	42	40.07	7.83	23	40.85	7.88	19	39.13	7.87	0.486	0.189		0.979	
Mid-Intervention	35	40.86	7.63	17	42.79	7.59	18	39.03	7.41	0.147		0.154		0.796
End-Intervention	39	38.88	7.70	21	38.90	7.90	18	38.86	7.68	0.986		0.111		0.927
Change		-1.19	6.61		-1.92	7.70		0.27	5.19	0.522				
Visceral Fat (L)														
Baseline	35	3.83	1.92	18	4.44	2.09	17	3.19	1.52	0.017*	<0.001**		<0.001**	
Mid-Intervention	33	4.65	2.75	17	5.15	2.91	16	4.11	2.55	0.127		0.255		0.289
End-Intervention	39	1.65*	1.07	21	1.54	0.62	18	1.78	1.44	0.945		<0.001**		0.001**
Change		-2.22	2.26		-3.05	1.87		-1.40	2.36	0.014*				

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference. All data presented as mean ± SD. * indicates significance ($p < 0.05$), ** indicates significance ($p < 0.017$). *Change* represents absolute change in variable from baseline to end-intervention. P^a p-values for comparing differences between participants in each diet group at the respective time point;

Characteristics	Total			Control (LFD)			Intervention (MedDiet)			P^a	P^b			
	n	mean	SD	n	mean	SD	n	mean	SD		LFD	P^c	MedDiet	P^d
independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values compares differences between baseline, mid-intervention and end-intervention timepoints in the control (LFD) group and P^d compares differences between baseline, mid-intervention and end-intervention timepoints in the intervention (MedDiet) group; one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative; post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test)($p < 0.017$).														

4.7.6 Effect of the LFD and MedDiet on Nutrient and Food Group Intake

Nutrient and food group intake (as mean values and standard deviation) are presented in **Table 4.4** and **Table 4.5**, respectively, for the overall cohort and LFD and MedDiet groups at baseline, mid-intervention and end-intervention. Mean change at the conclusion of the intervention for each variable is also presented. Nutrient and food group variables that did not demonstrate a significant change between timepoints are presented in **Supplementary Table 4**.

There were no significant differences in dietary intakes of participants assigned to MedDiet and LFD groups at baseline, except for higher intake of serve of legumes in the MedDiet group (p=0.030). Following the 12-week intervention, mean energy intake of both the LFD and MedDiet groups decreased by similar amounts (-1027.9 ± 2275.5 kJ, p =0.327, and -975.9 ± 2842.2 kJ, p =0.607, respectively), although neither of these reductions were significant. As a percentage of total energy consumed, protein intake remained unchanged in LFD and MedDiet groups. Carbohydrate intake as a percentage of total energy reduced significantly in the MedDiet group (43.7 ± 5.6% to 36.4 ± 7.6%, p =0.001) and remained unchanged in the LFD group (41.1 ± 7.7% to 40.9 ± 8.0%, p =0.703). Of particular importance was the change in the quality of carbohydrates consumed in the MedDiet group, wherein wholegrain intake increased markedly, albeit non-significantly, from baseline to mid intervention (1.4 ± 1.3 serves to 2.3 ± 1.6 serves, p =0.035) then decreased slightly and non-significantly from mid- to end-intervention (2.3 ± 1.6 serves to 1.8 ± 1.4 serves, p =0.157) and refined grain intake decreased significantly between baseline and end-intervention time points (6.4 ± 3.2 serves to 4.4 ± 4.3 serves, p =0.012). There was a slight, non-significant decrease in both groups for sugars as a percent of total energy consumed (LFD 16.9 ± 5.3% to 15.3 ± 4.2%, p =0.465, and MD 16.8 ± 7.4% to 14.1 ± 5.6%, p =0.092). There was a significant decrease in added sugars as a percent of total energy in the MedDiet group from baseline to end intervention (7.5 ± 6.8% to 4.6 ± 3.7%, p =0.034). Both the LFD and MedDiet group significantly decreased servings of added sugars from baseline to end-intervention (7.6 ± 7.3 serves to 4.1 ± 4.4 serves, p =0.047, and 10.4 ± 10.1 serves to 5.4 ± 5.1 serves, p =0.009, respectively).

As expected, total fat as a percentage of total energy significantly decreased in the LFD group (35.3 ± 6.9% to 32.6 ± 7.8%, p =0.047) and increased non-significantly in the MedDiet group (34.4 ± 5.6% to 40.0 ± 7.0%, p =0.092). Saturated fatty acids (SFAs) as a percent of total energy decreased non-significantly in both the LFD and MedDiet group (12.1 ± 3.9% to 10.3 ± 3.3%, p =0.088, and 11.8 ± 2.4% to 10.8 ± 3.3%, p =0.417). Monounsaturated fatty acids (MUFAs) as a percent of total energy decreased non-significantly in the LFD group (14.6 ± 3.7% to 13.7 ± 3.7%, p =0.092), while increasing significantly in the MedDiet group (14.2 ± 3.6% to 18.9 ± 4.8%, p =0.047). The MedDiet group were consuming significantly more MUFAs (%E) than the LFD group at the end of the intervention (18.9 ± 4.8% vs 13.7 ± 3.7%, p =0.0005, respectively). Polyunsaturated fatty acids (PUFAs) as a percent of total energy remained relatively unchanged in the LFD group (5.9 ± 2.5%

to 5.8 ± 1.9%, p =0.204) and increased non-significantly in the MedDiet group (5.8 ± 2.2% to 7.6 ± 2.4%, p =0.211). The MedDiet group were consuming significantly more PUFAs (%E) compared to the LFD group at the end of the intervention (7.6 ± 2.4% vs 5.8 ±1.9%, p =0.012, respectively). Trans fatty acids decreased in both LFD (1.3 ± 0.7 g to 0.9 ± 0.6 g, p =0.229) and MedDiet (1.2 ± 0.7 g to 0.9 ± 0.5 g, p =0.260) groups, albeit not significantly in either group. Very long chain omega-3 fatty acids increased non-significantly in the LFD group from baseline to end-intervention (0.3 ± 0.4 g to 0.5 ± 1.2 g, p =0.465), while the MedDiet group increased markedly, albeit non-significantly, from baseline to end-intervention (0.3 ± 0.4 g to 0.9 ± 0.9 g, p =0.066). The MedDiet group were consuming significantly more very long chain omega-3 fatty acids than the LFD group at mid-intervention (p =0.037) and end-intervention (p =0.005).

Similar trends were observed for DHA in the LFD and MedDiet groups. DHA increased non-significantly from baseline to end-intervention following the LFD (0.1 ± 0.2 g to 0.2 ± 0.5 g, p =0.449) and increased markedly, albeit non-significantly, from baseline to end-intervention in the MedDiet group (0.1 ± 0.2 g to 0.5 ± 0.6 g, p =0.069). DHA was significantly greater in the MedDiet group than the LFD group at mid-intervention (0.4 ± 0.3 g vs 0.2 ± 0.2 g, p =0.029) and end-intervention (0.5 ± 0.6g vs 0.2 ± 0.5g, p =0.006). EPA increased in both groups, though the change was only significant for the MedDiet group (0.1 ± 0.1 g to 0.3 ± 0.3 g, p =0.03) and not the LFD group (0.1 ± 0.1g to 0.2 ± 0.5g, p =0.943). At mid- and end-intervention timepoints, the MedDiet group had significantly higher EPA than the LFD group (0.3 ± 0.3 g vs 0.1 ± 0.1 g, p =0.047, and 0.3 ± 0.3 g vs 0.2 ± 0.5 g, p =0.008, respectively). DPA increased in both the LFD and MedDiet groups slightly and non-significantly (p =0.956 and p =0.141, respectively).

Changes for components of long chain n-3 fatty acids were consistent for food group intake; both the LFD and MedDiet group increased their intake of high long chain n-3 seafood serves, however this change was only significant in the MedDiet group (LFD 0.1 ± 0.2 serves to 0.2 ± 0.5 serves, p =0.349, and MD 0.1 ± 0.3 serves to 0.4 ± 0.5 serves, p =0.036, respectively). The MedDiet group were consuming significantly more high long chain n-3 seafood at end intervention than the LFD group (p =0.035). Oil equivalents, mostly composed of extra virgin olive oil (EVOO) in this cohort, decreased slightly in the LFD group (8.2 ± 4.8 serves to 7.5 ± 4.8 serves, p =0.662) and increased significantly in the MedDiet group (9.5 ± 6.7 serves to 13.4 ± 8.3 serves, p =0.030). At both mid- and end-intervention timepoints the MedDiet group were consuming significantly more oil equivalents than the LFD group (13.2 ± 8.5 serves vs 7.2 ± 3.5 serves, p =0.003, and 13.4 ± 8.3 serves vs 7.5 ± 4.8 serves, p =0.011, respectively).

Linoleic acid decreased in the LFD group (10.9 ± 6.9 g to 9.3 ± 6.4 g, p =0.838) and increased in the MedDiet (12.6 ± 8.3 g to 14.4 ± 8.7 g, p =0.678), however changes in both groups were non-significant. The MedDiet group had a significantly greater linoleic acid intake compared to the LFD group at mid-intervention (14.9 ± 7.4 g vs 9.4 ± 4.4 g, p =0.009) and end-intervention (14.4 ± 8.7 g vs 9.3 ± 6.4 g, p =0.037). This same trend was observed in tocopherol alpha and vitamin E. The

LFD decreased intake of tocopherol alpha and vitamin E, whereas the MedDiet increased intake of tocopherol alpha and vitamin E, both non-significantly. Tocopherol alpha and vitamin E were both significantly higher in the MedDiet group than the LFD group at mid- and end-intervention ($p < 0.05$). There was a significant reduction in calcium intake in both the LFD and MedDiet group from baseline to end of intervention, however this change was only significant in the LFD group (838.6 ± 359.6 mg to 645.6 ± 253.6 mg, $p = 0.025$, and 861.7 ± 466.6 mg to 780.2 ± 354.9 mg, $p = 0.801$, respectively).

Table 4.4. Nutrient intake variables at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

	Total		Control (LFD)		Intervention (MedDiet)		P[a]	P[b]			
	mean	SD	mean	SD	mean	SD		LFD	P[c]	MedDiet	P[d]
Macronutrients											
Energy (kJ)											
Baseline	8619.71	3160.72	8136.45	2752.66	9204.71	3582.57	0.397				
Mid-Intervention	8449.91	3217.85	8174.61	3607.69	8725.21	2853.48	0.308	0.327	0.711	0.607	0.616
End-Intervention	7654.08	3024.67	7032.55	2815.87	8379.21	3176.09	0.257		0.287		0.647
Change	-1003.87	2517.87	-1027.89	2275.45	-975.86	2842.21	0.950				
Protein (% of total E)											
Baseline	18.95	3.89	19.54	4.01	18.24	3.73	0.287				
Mid-Intervention	21.42	6.00	22.36	3.92	20.47	7.53	0.353	0.198	0.221	0.275	0.492
End-Intervention	20.51	4.68	20.95	5.10	20.00	4.22	0.537		0.901		0.526
Change	1.43	4.37	1.41	4.65	1.46	4.15	0.976				
Carbohydrate (% of total E)											
Baseline	42.27	6.94	41.09	7.76	43.70	5.67	0.23				
Mid-Intervention	39.00	8.44	41.51	6.13	36.50	9.78	0.076	0.703	0.243	0.001*	0.004**
End-Intervention	38.86	8.06	40.99	8.01	36.37	7.59	0.073		0.455		0.945
Change	-3.07	9.18	0.00	9.40	-6.65	7.70	0.022*				
Sugars (% of total E)											
Baseline	16.85	6.28	16.90	5.33	16.78	7.43	0.604				
Mid-Intervention	14.95	5.81	16.65	7.32	13.25	3.14	0.181	0.465	0.586	0.092	0.122
End-Intervention	14.77	4.87	15.35	4.21	14.11	5.60	0.364		0.906		0.744
Change	-2.19	6.35	-2.04	5.99	-2.37	6.92	0.874				
Added sugars (% of total E)											
Baseline	6.68	5.80	6.02	4.85	7.47	6.83	0.471				
Mid-Intervention	4.03	3.21	4.06	3.44	4.00	3.07	0.938	0.073	0.028	0.034*	0.028
End-Intervention	4.10*	3.11	3.65	2.49	4.62	3.72	0.530		0.463		0.446

	Total		Control (LFD)		Intervention (MedDiet)		P[a]	P[b]			
	mean	SD	mean	SD	mean	SD		LFD	P[c]	MedDiet	P[d]
Change	-2.76	4.36	-2.70	3.82	-2.83	5.03	0.552				
Total fat (% of total E)											
Baseline	34.91	6.31	35.33	6.95	34.39	5.56	0.658				
Mid-Intervention	34.78	7.97	30.70	5.58	38.87	8.03	0.003*	0.048*	0.005**	0.092	0.058
End-Intervention	36.07	8.25	32.66	7.83	40.05	7.00	0.003*		0.044		0.500
Change	0.99	9.08	-2.70	8.44	5.30	8.00	0.005*				
Saturated fat (% of total fat)											
Baseline	37.87	8.19	37.64	8.89	38.16	7.48	0.840				
Mid-Intervention	33.48	8.37	36.81	9.47	30.15	5.57	0.015	0.176	0.599	0.001*	0.009**
End-Intervention	32.19*	7.75	34.40	6.93	29.73	8.05	0.063		0.516		0.790
Change	-5.43	9.09	-2.94	10.90	-8.20	5.64	0.068				
Saturated fat (% of total E)											
Baseline	12.01	3.29	12.11	3.90	11.88	2.45	0.821				
Mid-Intervention	10.47	3.03	10.25	3.23	10.69	2.89	0.670	0.088	0.105	0.417	0.615
End-Intervention	10.52*	3.28	10.27	3.35	10.82	3.27	0.608		0.817		0.797
Change	-1.50	3.75	-1.83	4.14	-1.12	3.31	0.554				
Mono-unsaturated fat (% of total fat)											
Baseline	44.14	5.57	44.31	6.01	43.95	5.14	0.752				
Mid-Intervention	46.59	7.76	44.35	7.34	48.83	7.71	0.111	0.465	0.647	0.003*	0.018
End-Intervention	47.65*	5.66	45.57	3.85	49.96	6.52	0.026*		0.287		0.557
Change	3.24	6.28	0.80	6.37	5.95	5.07	0.010*				
Mono-unsaturated fat (% of total E)											
Baseline	14.43	3.64	14.58	3.77	14.25	3.57	0.810				
Mid-Intervention	15.27	5.26	12.64	3.31	17.90	5.60	0.005*	0.092	0.094	0.047*	0.018
End-Intervention	16.12*	4.97	13.68	3.69	18.96	4.82	<0.001*		0.446		0.011**
Change	1.58	5.39	-0.96	4.75	4.54	4.60	0.001*				

	Total		Control (LFD)		Intervention (MedDiet)		P[a]	P[b]			
	mean	SD	mean	SD	mean	SD		LFD	P[c]	MedDiet	P[d]
Poly-unsaturated fat (% of total fat)											
Baseline	17.98	6.11	18.06	6.82	17.89	5.30	0.870				
Mid-Intervention	19.93	5.15	18.84	4.45	21.02	5.68	0.462	0.465	0.396	0.311	0.145
End-Intervention	20.16	5.24	20.03	5.91	20.31	4.56	0.553		0.309		0.679
Change	2.19	8.24	2.14	8.85	2.25	7.75	0.967				
Poly-unsaturated fat (% of total E)											
Baseline	5.86	2.38	5.89	2.52	5.82	2.25	0.870				
Mid-Intervention	6.46	2.39	5.32	1.41	7.59	2.65	0.003*	0.204	0.472	0.211	0.085
End-Intervention	6.66	2.28	5.80	1.87	7.66	2.36	0.012*		0.227		0.983
Change	0.76	3.27	-0.07	3.20	1.72	3.15	0.088				
Dietary Fibre (g)											
Baseline	24.25	10.40	24.37	11.19	24.11	9.67	0.937				
Mid-Intervention	25.89	8.92	24.82	8.92	26.96	9.05	0.480	0.783	0.548	0.314	0.708
End-Intervention	24.19	9.84	24.01	10.57	24.41	9.23	0.903		0.734		0.591
Change	-0.06	10.82	-0.15	13.17	0.04	7.61	0.957				
Alcohol (g)											
Baseline	1.45	4.76	1.44	3.11	1.46	6.31	0.34				
Mid-Intervention	2.58	6.79	3.31	8.69	1.86	4.27	0.864	0.438	0.463	0.382	0.345
End-Intervention	2.24	5.23	3.03	5.33	1.37	5.14	0.303		0.917		0.499
Change	0.65	6.26	1.38	3.16	-0.17	8.54	0.455				
Linoleic Acid (g)											
Baseline	11.65	7.52	10.88	6.94	12.58	8.27	0.604				
Mid-Intervention	12.18	6.63	9.38	4.38	14.98	7.40	0.009*	0.838	0.647	0.678	0.306
End-Intervention	11.65	7.90	9.26	6.39	14.44	8.74	0.037*		0.981		0.983
Change	-0.14	7.78	-1.53	8.48	1.48	6.76	0.233				
ALA (g)											

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
Baseline	1.63	1.08	1.52	1.16	1.76	0.99	0.300				
Mid-Intervention	1.42	0.85	1.15	0.59	1.69	0.99	0.152	0.327	0.286	0.846	0.711
End-Intervention	1.48	0.77	1.32	0.78	1.67	0.74	0.088		0.309		0.983
Change	-0.11	1.12	-0.09	1.23	-0.14	1.02	0.882				
EPA (g)											
Baseline	0.08	0.13	0.09	0.14	0.07	0.12	0.622				
Mid-Intervention	0.19	0.25	0.10	0.13	0.28	0.30	0.047*	0.943	0.777	0.030*	0.005**
End-Intervention	0.23	0.39	0.19	0.47	0.28	0.28	0.008*		0.925		0.777
Change	0.15	0.34	0.09	0.37	0.21	0.32	0.305				
DPA (g)											
Baseline	0.06	0.05	0.07	0.05	0.06	0.06	0.235				
Mid-Intervention	0.09	0.09	0.07	0.06	0.11	0.11	0.203	0.956	0.777	0.141	0.118
End-Intervention	0.11	0.17	0.11	0.21	0.12	0.12	0.112		0.906		0.811
Change	0.05	0.16	0.04	0.18	0.06	0.14	0.756				
DHA (g)											
Baseline	0.14	0.20	0.13	0.20	0.15	0.21	0.733				
Mid-Intervention	0.27	0.31	0.17	0.24	0.37	0.34	0.029*	0.449	0.246	0.069	0.025
End-Intervention	0.33	0.54	0.21	0.48	0.48	0.59	0.006*		0.756		0.948
Change	0.19	0.51	0.08	0.37	0.33	0.63	0.139				
Very Long Chain Omega-3 Fatty Acids (g)											
Baseline	0.28	0.35	0.28	0.36	0.27	0.35	0.870				
Mid-Intervention	0.55	0.62	0.33	0.41	0.76	0.72	0.037*	0.465	0.679	0.066	0.018
End-Intervention	0.67	1.06	0.50	1.16	0.87	0.92	0.005*		0.586		0.913
Change	0.39	0.95	0.22	0.91	0.59	0.99	0.223				
Trans Fatty Acids (g)											
Baseline	1.26	0.73	1.29	0.77	1.24	0.70	0.870	0.229		0.260	
Mid-Intervention	1.08	0.79	1.07	0.86	1.09	0.73	0.943		0.984		1.000

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
End-Intervention	0.90	0.57	0.90	0.63	0.91	0.51	0.587		0.260		0.368
Change	-0.37	0.84	-0.40	0.82	-0.33	0.90	0.806				
Micronutrients											
Vitamin C (mg)											
Baseline	118.33	140.03	137.58	170.75	95.02	89.32	0.658				
Mid-Intervention	103.67	80.73	111.84	98.40	95.51	59.97	0.839	0.465	0.948	0.946	0.845
End-Intervention	102.19	84.21	107.29	91.70	96.24	76.73	0.945		0.463		0.983
Change	-18.37	172.80	-34.59	207.53	0.56	123.98	0.534				
Vitamin E (mg)											
Baseline	13.27	7.42	12.90	7.23	13.72	7.81	0.677				
Mid-Intervention	14.51	8.76	11.74	6.86	17.28	9.72	0.024*	0.662	0.586	0.092	0.102
End-Intervention	14.06	7.41	11.07	5.51	17.55	7.95	0.003*		0.586		0.711
Change	0.53	8.03	-2.08	9.18	3.56	5.19	0.027*				
Tocopherol Alpha (mg)											
Baseline	11.68	5.99	11.35	5.37	12.07	6.80	0.830				
Mid-Intervention	12.64	7.74	9.81	5.61	15.48	8.66	0.008*	0.838	0.306	0.311	0.064
End-Intervention	12.07	6.23	9.81	4.98	14.70	6.62	0.007*		0.906		0.811
Change	0.17	6.13	-1.76	6.65	2.41	4.69	0.032*				
Calcium (mg)											
Baseline	849.04	406.33	838.60	359.60	861.68	466.60	0.990				
Mid-Intervention	965.03	1277.43	1087.17	1794.04	842.89	330.87	0.214	0.025*	0.094	0.801	0.711
End-Intervention	707.70	307.95	645.57	253.57	780.18	354.97	0.148		0.619		0.711
Change	-155.05	406.39	-205.13	326.44	-96.64	486.91	0.413				

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; ALA, alpha linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. All data presented as mean ± SD. * indicates significance ($p < 0.05$), ** indicates significance ($p < 0.017$). *Change* represents absolute change in variable from baseline to end-intervention. P^a p-values for comparing differences between participants in each diet group at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values compares differences between baseline, mid-

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
intervention and end-intervention timepoints in the control (LFD) group and P^d compares differences between baseline, mid-intervention and end-intervention timepoints in the intervention (MedDiet) group; one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative; post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test)($p < 0.017$).											

Table 4.5. Mean food group intake at baseline, 6-week and 12-week timepoints in the overall cohort and within the LFD and MedDiet groups

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
Grains											
Baseline	6.93	3.07	6.14	2.92	7.89	3.04	0.075				
Mid-Intervention	6.53	3.36	6.26	2.68	6.79	3.99	0.696	0.204	0.184	0.066	0.157
End-Intervention	5.87	3.71	5.58	3.46	6.20	4.06	0.626		0.124		0.306
Change	-0.92	3.29	-0.25	3.48	-1.70	2.95	0.172				
Wholegrains											
Baseline	1.28	1.25	1.14	1.18	1.44	1.35	0.577				
Mid-Intervention	2.16	1.53	1.98	1.45	2.35	1.62	0.628	0.299	0.055	0.154	0.035*
End-Intervention	2.00*	1.83	2.16	2.13	1.82	1.43	0.728		0.309		0.157
Change	0.75	1.92	1.05	2.28	0.40	1.39	0.301				
Refined Grains											
Baseline	5.66	2.90	5.00	2.54	6.45	3.17	0.250				
Mid-Intervention	4.36	3.34	4.28	2.63	4.45	4.00	0.696	0.790	0.811	0.012*	0.050*
End-Intervention	3.86*	3.67	3.42	3.08	4.38	4.29	0.686		0.246		0.811
Change	-1.67	3.26	-1.30	3.27	-2.10	3.29	0.454				
Vegetables											
Baseline	3.78	2.61	4.20	2.92	3.27	2.14	0.257				
Mid-Intervention	3.29	1.84	3.40	1.95	3.17	1.78	0.720	0.939	0.691	0.911	0.665
End-Intervention	3.31	1.96	3.28	2.18	3.34	1.74	0.920		0.732		0.782

	Total		Control (LFD)		Intervention (MedDiet)		P[a]	P[b]			
	mean	SD	mean	SD	mean	SD		LFD	P[c]	MedDiet	P[d]
Change	-0.50	3.38	-0.88	3.98	-0.05	2.54	0.455				
Dark Green Vegetables											
Baseline	0.57	1.54	0.64	1.98	0.48	0.78	0.260		0.616		0.811
Mid-Intervention	0.26	0.28	0.25	0.31	0.26	0.26	0.839	0.881	0.619	0.948	0.586
End-Intervention	0.40	0.67	0.36	0.71	0.45	0.63	0.900				
Change	-0.20	1.74	-0.33	2.19	-0.06	1.03	0.880				
Tomatoes (serve)											
Baseline	0.39	0.44	0.51	0.49	0.24	0.32	0.052		0.043		0.088
Mid-Intervention	0.33	0.29	0.27	0.31	0.39	0.28	0.265	0.021*	0.876	0.010*	0.023*
End-Intervention	0.41	0.45	0.26	0.37	0.58	0.49	0.014*				
Change	0.04	0.52	-0.21	0.43	0.33	0.47	0.050*				
Fruit											
Baseline	1.34	1.24	1.60	1.44	1.03	0.87	0.289				
Mid-Intervention	1.31	1.16	1.54	1.31	1.09	0.97	0.501	0.252	0.407	0.684	0.758
End-Intervention	1.16	1.17	1.32	1.35	0.97	0.92	0.646		0.717		0.472
Change	-0.17	1.38	-0.34	1.59	-0.03	1.11	0.413				
Red Meats											
Baseline	0.79	0.97	0.80	0.81	0.77	1.16	0.617				
Mid-Intervention	0.95	1.02	1.13	1.08	0.78	0.95	0.406	0.223	0.256	0.672	0.906
End-Intervention	0.50	0.57	0.58	0.56	0.40	0.57	0.192		0.019*		0.158
Change	-0.29	1.07	-0.19	0.76	-0.42	1.36	0.507				
Poultry											
Baseline	0.52	0.50	0.51	0.58	0.54	0.39	0.454				
Mid-Intervention	0.57	0.73	0.74	0.77	0.40	0.67	0.203	0.362	0.381	0.074	0.049
End-Intervention	0.66	0.85	0.65	0.67	0.67	1.04	0.728		0.535		0.532
Change	0.10	0.98	0.10	0.82	0.10	1.16	0.982				
Seafood											

	Total		Control (LFD)		Intervention (MedDiet)		P[a]	P[b]			
	mean	SD	mean	SD	mean	SD		LFD	P[c]	MedDiet	P[d]
High long chain omega-3											
Baseline	0.10	0.27	0.09	0.22	0.11	0.33	0.568				
Mid-Intervention	0.26	0.38	0.14	0.25	0.38	0.45	0.226	0.349	0.285	0.036*	0.055
End-Intervention	0.32*	0.54	0.21	0.55	0.44	0.50	0.035*		0.799		0.625
Change	0.21	0.49	0.11	0.41	0.33	0.57	0.172				
Legumes											
Baseline	0.24	0.70	0.17	0.79	0.32	0.57	0.030*				
Mid-Intervention	0.38	0.66	0.38	0.76	0.38	0.57	0.864	0.159	0.167	0.574	0.583
End-Intervention	0.24	0.49	0.25	0.57	0.23	0.40	0.835		0.875		0.182
Change	-0.02	0.50	0.06	0.44	-0.11	0.57	0.297				
Nuts											
Baseline	0.69	1.20	0.54	0.98	0.87	1.44	0.41				
Mid-Intervention	0.72	1.08	0.41	0.59	1.04	1.35	0.017*	0.189	0.717	0.074	0.124
End-Intervention	0.67*	1.02	0.36	0.51	1.03	1.32	0.156		0.820		0.795
Change	-0.04	1.03	-0.18	1.07	0.12	0.99	0.376				
Dairy											
Baseline	1.66	1.11	1.60	0.96	1.72	1.29	0.870				
Mid-Intervention	1.99	4.32	2.58	6.06	1.40	1.02	0.815	0.327	0.170	0.678	0.267
End-Intervention	1.25	0.75	1.20	0.70	1.30	0.81	0.686		0.943		0.446
Change	-0.43	1.12	-0.42	0.85	-0.45	1.40	0.941				
Milk											
Baseline	0.99	0.90	0.85	0.77	1.16	1.03	0.230				
Mid-Intervention	1.48	4.38	2.11	6.16	0.84	0.82	0.791	0.521	0.653	0.412	0.381
End-Intervention	0.76	0.54	0.73	0.45	0.79	0.65	0.813		0.796		0.744
Change	-0.25	0.96	-0.12	0.67	-0.39	1.22	0.395				
Yoghurt											
Baseline	0.17	0.40	0.23	0.49	0.10	0.22	0.488	0.784		0.066	

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
Mid-Intervention	0.21	0.32	0.15	0.26	0.27	0.36	0.542		0.878		0.017**
End-Intervention	0.19	0.26	0.15	0.23	0.24	0.30	0.443		0.767		0.678
Change	0.01	0.48	-0.10	0.56	0.13	0.33	0.133				
Oil Equivalents											
Baseline	8.82	5.70	8.22	4.79	9.54	6.70	0.686				
Mid-Intervention	10.17	7.09	7.18	3.49	13.17	8.51	0.003*	0.662	0.711	0.030*	0.058
End-Intervention	10.23*	7.20	7.49	4.88	13.41	8.25	0.011*		0.586		0.811
Change	1.34	5.62	-0.61	6.19	3.61	3.93	0.041*				
Solid fat Equivalents											
Baseline	8.84	5.14	8.35	4.84	9.44	5.55	0.714				
Mid-Intervention	7.19	4.40	7.42	5.12	6.97	3.69	0.988	0.465	0.231	0.411	0.145
End-Intervention	6.29	3.82	6.20	3.82	6.40	3.93	1.000		0.831		0.528
Change	-2.65	5.08	-2.13	3.87	-3.25	6.28	0.500				
Added Sugars											
Baseline	8.85	8.68	7.57	7.28	10.40	10.10	0.283				
Mid-Intervention	5.40	5.19	5.07	4.69	5.73	5.76	0.864	0.047*	0.039*	0.009*	0.028*
End-Intervention	4.72*	4.71	4.10	4.36	5.44	5.11	0.223		0.309		0.845
Change	-4.46	6.07	-3.89	4.60	-5.12	7.53	0.323				
Alcoholic Beverages											
Baseline	0.15	0.48	0.14	0.31	0.15	0.63	0.34				
Mid-Intervention	0.26	0.68	0.33	0.87	0.19	0.43	0.815	0.341	0.398	0.382	0.345
End-Intervention	0.22	0.52	0.29	0.52	0.14	0.51	0.349		0.866		0.499
Change	0.06	0.62	0.13	0.31	-0.02	0.86	0.454				

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation. All data presented as mean ± SD. * indicates significance ($p < 0.05$), ** indicates significance ($p < 0.017$). *Change* represents absolute change in variable from baseline to end-intervention. P^a p-values for comparing differences between participants in each diet group at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values compares differences between baseline, mid-intervention and end-intervention timepoints in the control (LFD) group and P^d compares differences between baseline, mid-

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
intervention and end-intervention timepoints in the intervention (MedDiet) group; one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative; post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test)($p < 0.017$).											

4.7.7 Dietary intakes in the LFD and MedDiet study arms at end of intervention (12-weeks) compared to prescribed dietary interventions

At 12-weeks, participants in the LFD arm were partially meeting the estimated Acceptable Macronutrient Distribution Ranges (AMDR) related to reduced risk of chronic disease as outlined in the Australian Guide to Healthy Eating.[356] Total energy intake from fat was slightly above the recommended intake (actual 32.7 ± 7.8% vs prescribed 30%), total energy intake from carbohydrate was lower than the recommended intake (actual 41 ± 8.0% vs prescribed 50%) and total energy intake from protein met the recommended intake amount (actual 20.9 ± 5.1% vs prescribed 20%).

Table 4.6 contains the nutrient composition of the Australian Mediterranean Diet which was prescribed to study participants who were randomised to the MedDiet group (refer to section 2.7.1), compared to the actual nutrient intake of the MedDiet group at 12-weeks. The MedDiet arm were consuming almost 1MJ less in total energy intake (8.4 ± 3.2MJ) than the prescribed amount (9.4MJ). Participants were consuming a slightly more carbohydrate as a percentage of total energy (36.4 ± 7.6%) than prescribed (33.8%), as well as a higher amount of protein as a percentage of total energy (20 ± 4.2%) than the prescribed diet (15.8%). They were also consuming more SFA (10.8 ± 3.3%E) than the prescribed amount (8.9%E). Alternatively, participants were consuming slightly less added sugar (4.6 ± 3.7%E) and total fat (40.1 ± 7.0%E) than the prescribed diet plan (5.2%E and 41.8%E, respectively), as well as less MUFA (19 ± 4.8%E) and PUFA (7.6 ± 2.4%E) than prescribed (22.3%E and 10.6%E, respectively) and not meeting the recommended amount of fibre per day (24.4 ± 9.2g vs 41.1g). Actual intakes of linoleic acid, ALA and total LCN3s did not meet the recommendations prescribed in the MedDiet intervention.

Table 4.6. Daily nutrient intake profile of the prescribed Mediterranean Diet and actual nutrient intake of participants in the MedDiet arm of the MEDINA Study at 12-weeks.

Nutrients	Australian Mediterranean Composition[529]	Daily intake of MedDiet study group
Energy (MJ)	9.4	8.4
Protein (%E)	15.8	20
CHO (%E)	33.8	36.4
Added sugar (%E)	5.2	4.6
Total fat (%E)	41.8	40.1
SFA (%E)	8.9	10.8
MUFA (%E)	22.3	19
PUFA (%E)	10.6	7.6
Alcohol (%E)	2.4	-
Fibre (g/d)	41.1	24.4
Linoleic acid n-6 (g)	18.7	14.4
α linolenic acid n-3 (g)	4.9	1.7
Total LCN3s (mg)	932	870

Abbreviations: MedDiet, Mediterranean Diet; CHO, carbohydrates; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LCN3s, long chain omega 3 fatty acids.

4.7.8 Correlations

Partial correlation was used to assess the linear relationship between inflammatory markers; hs-CRP, TNF-α, IL-6, Adiponectin, Leptin and Resistin, and liver outcomes, HOMA-IR, biochemistry, anthropometry and nutrient and food group intake in each diet group, after controlling for weight (kg) change between baseline and end intervention.

Appendix 11; Supplementary Table 5 presents correlation coefficients between inflammatory markers and other inflammatory markers at the end-intervention timepoint. hs-CRP was significantly ($p<0.05$), moderately and positively correlated with circulating levels of resistin ($r =0.45$). There were no significant associations between any other inflammatory cytokines or adipokines.

4.7.8.1 Association between inflammatory markers and liver outcomes, biochemical, anthropometry and body composition variables in each diet group

Correlation coefficients for each inflammatory marker and measures of IHL, LSM, HOMA-IR, biochemistry, anthropometry and body composition at end intervention in each diet group are presented in **Table 4.7**. There were no significant associations between any of the inflammatory markers and IHL or LSM in either diet group at end-intervention. In the LFD group, hs-CRP had a significant ($p<0.05$) strong, positive correlation with NC ($r =0.56$) and moderate, positive correlations with GGT and glucose ($r =0.50$ and 0.49, respectively). In the MedDiet group, hs-CRP was significantly ($p <0.05$) strongly and positively correlated with HOMA-IR, fasting insulin and FM ($r =0.80$, 0.73 and 0.63, respectively).

In the LFD group, TNF-α had a significant ($p <0.05$) strong, positive correlation with fasting insulin ($r =0.52$) and a strong negative correlation with HDL, LDL and total cholesterol ($r =0.63$, 0.59 and 0.59, respectively). There were no significant correlations between TNF-α with biochemical, anthropometric or body composition measures for the MedDiet arm.

IL-6 was significantly ($p <0.05$), moderately and positively correlated with GGT ($r =0.47$) in the LFD group, and was significantly ($p <0.05$), strongly and positively correlated with AST and ALT in the MedDiet group ($r =0.60$ and 0.55, respectively).

In the LFD group, adiponectin was a significantly ($p <0.05$), moderately and positively correlated with HDL ($r =0.45$). In the MedDiet group, adiponectin was significantly ($p <0.05$), strongly and positively correlated with HC, BMI, WC, FM, body weight and VF ($r =0.85$, 0.78, 0.70, 0.70, 0.65 and 0.52, respectively).

In the LFD group, leptin was significantly ($p < 0.05$), strongly and positively correlated with fasting insulin and FM ($r = 0.55$ and 0.55, respectively). In the MedDiet group, leptin was significantly ($p < 0.05$), strongly and positively correlated with HDL, HC and FM ($r = 0.61$, 0.58 and 0.51, respectively), while strongly and negatively associated with WHR ($r = -0.53$) and moderately, negatively associated with glucose ($r = -0.49$).

In the LFD group, resistin was significantly ($p < 0.05$), strongly and positively correlated with NC ($r = 0.77$), and strongly and negatively correlated with systolic and diastolic BP ($r = -0.57$ and -0.71, respectively). In the MedDiet group, resistin was significantly ($p < 0.05$), strongly and positively correlated with HDL and GGT ($r = 0.57$ and 0.53, respectively).

Table 4.7. Partial correlations between inflammatory markers with liver, biochemical, anthropometric and body composition variables in each diet group at end intervention, values adjusted for mean weight-loss

Correlation Coefficients (adjusted for weight change)							
Outcome		**hs-CRP**	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Liver outcomes and biochemistry							
IHL (%)	LFD	0.36	0.37	0.15	-0.11	0.25	0.05
	MedDiet	-0.20	0.03	-0.12	0.19	0.06	-0.29
LSM (kPa)	LFD	0.41	0.14	0.39	-0.12	0.26	0.44
	MedDiet	0.43	-0.03	-0.08	0.44	-0.14	0.36
Glucose (mmol/L)	LFD	0.49*	0.32	0.29	-0.28	-0.01	0.24
	MedDiet	0.02	-0.03	-0.14	-0.30	-0.50*	-0.14
Insulin (mIU/L)	LFD	0.32	0.52*	0.09	-0.30	0.55*	0.24
	MedDiet	0.73**	-0.28	-0.11	0.38	-0.27	0.46
HOMA-IR	LFD	0.42	0.48*	0.19	-0.37	0.38	0.26
	MedDiet	0.80**	-0.30	-0.18	0.34	-0.38	0.47
ALT (U/L)	LFD	0.41	0.07	0.34	0.15	0.21	0.09
	MedDiet	-0.36	0.00	0.55*	-0.14	-0.09	0.03
AST (U/L)	LFD	0.36	0.31	0.35	0.11	0.05	-0.06
	MedDiet	-0.23	-0.10	0.60*	-0.16	-0.16	0.15
GGT (U/L)	LFD	0.50*	-0.37	0.47*	0.15	0.35	0.11
	MedDiet	0.43	-0.41	0.27	0.20	-0.17	0.53*
Cholesterol (mmol/L)	LFD	-0.25	-0.58**	-0.23	0.30	0.23	-0.31
	MedDiet	0.17	-0.20	0.42	0.06	0.28	0.14
HDL (mmol/L)	LFD	-0.30	-0.63**	0.14	0.45*	0.15	-0.21
	MedDiet	0.54	-0.39	0.23	0.41	0.61**	0.57*
LDL (mmol/L)	LFD	-0.27	-0.59**	-0.28	0.24	0.14	-0.33
	MedDiet	0.16	-0.11	0.34	0.02	0.26	0.10
Triglycerides (mmol/L)	LFD	0.23	0.37	0.03	-0.04	0.27	0.08

Correlation Coefficients (adjusted for weight change)							
Outcome		**hs-CRP**	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
	MedDiet	-0.17	-0.24	0.48	-0.11	-0.13	-0.19
Anthropometry and body composition							
Weight (kg)	LFD	0.37	0.24	0.12	-0.20	0.10	0.15
	MedDiet	0.07	0.25	-0.21	0.65**	0.27	0.17
BMI (kg/m2)	LFD	0.38	0.09	0.32	-0.13	0.29	-0.01
	MedDiet	0.41	0.11	-0.32	0.78**	0.41	0.27
WC (cm)	LFD	0.33	0.22	0.18	-0.25	0.30	0.07
	MedDiet	0.30	0.12	-0.24	0.70**	0.40	0.21
HC (cm)	LFD	0.14	0.30	0.06	-0.15	0.41	0.05
	MedDiet	0.49	0.14	-0.29	0.85**	0.58*	0.37
WHR	LFD	0.37	-0.32	0.33	-0.28	-0.16	0.01
	MedDiet	-0.16	0.03	0.10	-0.32	-0.53*	-0.21
NC (cm)	LFD	0.56*	0.16	0.03	-0.28	-0.05	0.77**
	MedDiet	0.10	0.04	0.12	0.07	-0.23	0.22
Fat mass (%)	LFD	-0.05	0.01	0.26	0.32	0.55*	-0.24
	MedDiet	0.63*	-0.19	-0.40	0.70**	0.51*	0.31
Visceral Fat (l)	LFD	0.45	-0.15	0.19	-0.02	-0.04	0.12
	MedDiet	-0.25	0.26	-0.06	0.52*	0.39	0.05
Blood Pressure (Systolic)	LFD	-0.49*	-0.17	-0.02	0.10	-0.11	-0.57*
	MedDiet	-0.13	-0.06	-0.05	-0.11	-0.19	-0.27
Blood Pressure (Diastolic)	LFD	-0.39	-0.11	0.02	0.01	0.01	-0.71**
	MedDiet	0.12	0.01	-0.06	0.40	0.40	0.34

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.

hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IHL, Intrahepatic Lipid; LSM, Liver Stiffness Measure; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

4.7.8.2 Association between inflammatory markers and nutrient and food group intake

Correlation coefficients for each inflammatory marker associated with nutrient and food group intake parameters at the end-intervention timepoint in each diet group are presented in **Table 4.8**.

In the LFD group, there were no significant correlations between hs-CRP, TNF-α, IL-6, adiponectin or resistin with any of the nutrient or food group variables. In the LFD group, leptin was significantly ($p < 0.05$), strongly and positively associated with SFA (% total fat) ($r = 0.75$) and moderately and positively correlated with SFA (%E) ($r = 0.48$). Leptin was also significantly ($p < 0.05$), strongly and negatively correlated with PUFA (% total fat), PUFA (%E) and oil equivalents ($r = -0.59$, -0.51 and -0.54) and moderately and negatively associated with ALA, potassium, magnesium, fruit, tocopherol alpha, vitamin E and energy (kJ) ($r = -0.52$, -0.49, -0.48, -0.48, -0.46, -0.46 and -0.45).

In the MedDiet group, hs-CRP was significantly ($p < 0.05$), strongly and positively correlated with protein (%E) and serves of vegetables ($r = 0.57$ and 0.56, respectively). In the MedDiet group, there were significant ($p < 0.05$), strong and positive correlations between TNF-α and tocopherol alpha, PUFA (%E), linoleic acid, vitamin E, oil equivalents, MUFA (%E) and total fat (%E) ($r = 0.60$, 0.59, 0.57, 0.55, 0.52, 0.51 and 0.50). TNF-α was also significantly ($p < 0.05$), moderately and negatively correlated with saturated fat (% total fat) ($r = -0.49$). In the MedDiet arm, IL-6 was significantly ($p < 0.05$), strongly and positively correlated with added sugars (%E), added sugars (serves), saturated fat (%E), saturated fat (% total fat), total sugars (%E) and serves of solid fat equivalents ($r = 0.78$, 0.78, 0.70, 0.68, 0.63 and 0.55) and moderately and positively correlated with serves of milk ($r = 0.49$). IL-6 was also significantly ($p < 0.01$), strongly and negatively correlated with MUFA (% total fat) in the MedDiet group ($r = -0.62$). In the MedDiet group, adiponectin was significantly ($p < 0.05$), moderately and negatively associated with serves of refined grains ($r = -0.48$). Leptin was significantly ($p < 0.01$), strongly and positively correlated with total sugars (%E) ($r = 0.70$) in the MedDiet group, and strongly and negatively correlated with grains, refined grains, EPA, DPA, DHA and serves of seafood high in long chain omega-3 fatty acids ($r = -0.50$, -0.56, -0.55, -0.52, -0.50 and -0.53). There were no significant correlations between resistin with any nutrient or food group variables for the MedDiet group. Correlations were conducted for additional nutrient and food group intake variables (e.g. fibre, calcium, iron, zinc, whole grains, vegetables and red meat), though they did not demonstrate a significant relationship therefore the data is not shown within these results.

Table 4.8. Partial correlations between inflammatory markers with nutrient and food group intake in each diet group at end intervention, values adjusted for mean weight-loss

		Correlation Coefficients (adjusted for weight change)					
		hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Nutrients							
Energy (kJ)	LFD	-0.41	-0.05	-0.18	-0.22	-0.45*	-0.10
	MedDiet	-0.40	0.39	-0.04	-0.37	-0.40	-0.16
Protein (%E)	LFD	0.46	0.07	0.31	0.20	0.10	0.04
	MedDiet	0.57*	-0.12	-0.37	0.30	-0.25	0.10
Carbohydrate (%E)	LFD	-0.05	0.08	0.00	-0.07	-0.13	0.11
	MedDiet	-0.43	-0.33	0.29	-0.39	0.07	-0.12
Sugars (%E)	LFD	0.00	0.09	-0.05	-0.42	-0.07	0.04
	MedDiet	-0.06	-0.19	0.63**	0.06	0.70**	0.24
Added Sugars (%E)	LFD	-0.22	-0.10	-0.02	-0.23	-0.11	-0.13
	MedDiet	-0.30	-0.11	0.78**	-0.05	0.31	0.14
Total fat (%E)	LFD	-0.39	-0.03	-0.32	-0.06	0.08	-0.08
	MedDiet	0.02	0.50*	-0.02	0.20	0.02	0.03
Saturated fat (% of total fat)	LFD	0.33	0.13	0.29	0.30	0.75**	0.21
	MedDiet	0.12	-0.49*	0.68**	0.14	0.24	0.25
Saturated fat (%E)	LFD	-0.20	0.00	-0.14	0.12	0.48*	0.02
	MedDiet	0.07	-0.15	0.70**	0.24	0.24	0.21
Mono-unsaturated fat (% of total fat)	LFD	-0.18	-0.12	-0.18	-0.04	-0.44	-0.02
	MedDiet	-0.02	0.34	-0.62**	-0.20	-0.22	-0.23
Mono-unsaturated fat (%E)	LFD	-0.39	-0.03	-0.34	-0.08	-0.11	-0.05
	MedDiet	-0.01	0.51*	-0.36	-0.01	-0.12	-0.12
Poly-unsaturated fat (% of total fat)	LFD	-0.27	-0.07	-0.22	-0.33	-0.59**	-0.23
	MedDiet	-0.20	0.41	-0.35	0.04	-0.11	-0.11
Poly-unsaturated fat (%E)	LFD	-0.45	0.00	-0.32	-0.31	-0.51*	-0.16
	MedDiet	-0.17	0.59*	-0.29	0.14	-0.04	-0.05
Linoleic Acid	LFD	-0.36	-0.14	-0.15	-0.29	-0.44	-0.14
	MedDiet	-0.31	0.57*	-0.22	-0.14	-0.26	-0.08
ALA	LFD	-0.28	0.10	-0.15	-0.33	-0.52*	-0.16
	MedDiet	-0.18	0.40	-0.21	-0.10	-0.06	0.10
EPA	LFD	-0.09	0.38	-0.14	0.10	-0.29	-0.11
	MedDiet	-0.09	0.03	0.06	0.04	-0.55*	0.10
DPA	LFD	-0.08	0.40	-0.14	0.09	-0.29	-0.10
	MedDiet	0.27	-0.17	-0.05	0.13	-0.52*	0.12
DHA	LFD	-0.09	0.40	-0.11	0.12	-0.29	-0.10
	MedDiet	-0.29	0.03	0.19	-0.21	-0.50*	-0.17

Correlation Coefficients (adjusted for weight change)							
		hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Very Long Chain Omega-3 FAs	LFD	-0.09	0.39	-0.13	0.11	-0.29	-0.10
	MedDiet	-0.19	0.00	0.13	-0.11	-0.56*	-0.06
Vitamin E	LFD	-0.32	0.02	-0.17	-0.17	-0.46*	-0.16
	MedDiet	-0.29	0.55*	-0.19	-0.23	-0.25	-0.05
Tocopherol Alpha	LFD	-0.30	0.06	-0.15	-0.17	-0.46*	-0.14
	MedDiet	-0.29	0.60*	-0.32	-0.20	-0.28	-0.11
Potassium	LFD	-0.29	0.02	-0.10	-0.14	-0.49*	-0.12
	MedDiet	-0.36	0.36	-0.12	-0.30	-0.28	-0.21
Magnesium	LFD	-0.37	0.00	-0.09	-0.18	-0.48*	-0.08
	MedDiet	-0.23	0.37	-0.18	-0.15	-0.18	0.06
Food Groups							
Grains	LFD	-0.21	0.00	-0.04	0.02	-0.34	-0.07
	MedDiet	-0.41	0.21	-0.19	-0.45	-0.50*	-0.20
Refined Grains	LFD	-0.02	0.15	-0.06	-0.11	-0.21	0.09
	MedDiet	-0.41	0.32	-0.11	-0.48*	-0.56*	-0.14
Vegetables	LFD	-0.20	0.05	-0.10	-0.28	-0.18	-0.20
	MedDiet	-0.56*	0.46	-0.02	-0.25	-0.16	-0.42
Fruit	LFD	-0.35	-0.07	-0.29	-0.27	-0.48*	-0.01
	MedDiet	0.19	0.07	-0.11	-0.06	0.12	0.37
Seafood (High LCN3)	LFD	-0.09	0.40	-0.12	0.12	-0.29	-0.12
	MedDiet	-0.28	-0.08	0.27	-0.22	-0.53*	0.00
Milk	LFD	0.14	0.49*	0.38	0.06	-0.04	0.06
	MedDiet	-0.03	-0.34	0.49*	-0.20	0.11	-0.12
Oil equivalents	LFD	-0.43	-0.04	-0.20	-0.19	-0.54*	-0.24
	MedDiet	-0.30	0.52*	-0.33	-0.27	-0.39	-0.21
Solid fat equivalents	LFD	-0.26	-0.09	-0.17	-0.20	0.04	0.08
	MedDiet	-0.05	-0.03	0.55*	-0.04	0.03	0.17
Added sugars (serve)	LFD	-0.22	-0.10	-0.02	-0.23	-0.11	-0.13
	MedDiet	-0.30	-0.11	0.78**	-0.05	0.31	0.14

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations. hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; ALA, Alpha Linolenic Acid; EPA, Eicosapentaenoic Acid; DPA, Docosapentaenoic Acid; DHA, Docosahexaenoic Acid; FAs, Fatty Acids; CHO, carbohydrate; LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; LCN3, long chain omega-3 fatty acids.

4.7.9 Diet Quality

4.7.9.1 Adherence to the Low-Fat Diet vs. adherence to the Mediterranean Diet – the PREDIMED Score

A validated, 14-item PREDIMED checklist was used to assess adherence to a MedDiet. In this section the results of MedDiet adherence obtained using this checklist within the MedDiet group are reported from baseline to end-intervention. Adherence to a MedDiet significantly increased from pre- to post-intervention (6.47 ± 1.98 to 9.17 ± 1.89, p =0.001) in the MedDiet group.

A separate 9-item checklist was developed by the PREDIMED study group to assess adherence to a LFD. In this section results of adherence to a LFD in the LFD group, at baseline and end-intervention timepoints, is reported. Adherence to a LFD increased from pre- to post-intervention, albeit not significantly (5.39 ± 2.04 to 6.35 ± 2.25, p =0.081) in the LFD group. **Figure 4.2** presents the results of overall adherence to each respective diet.

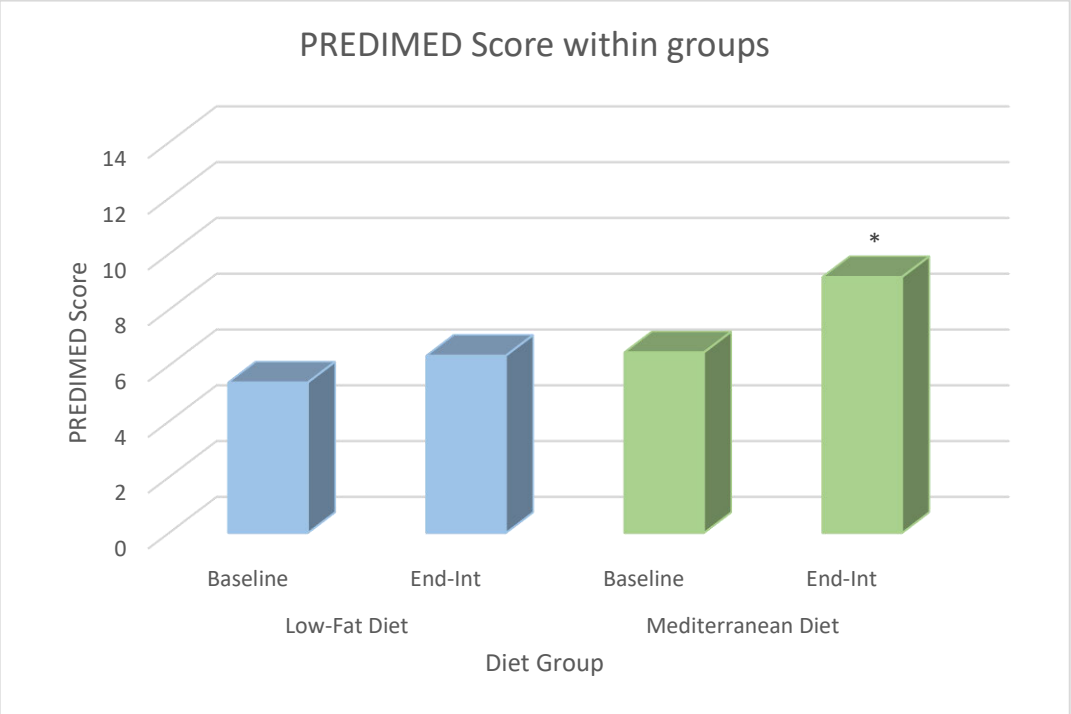


Figure 4.2. PREDIMED Checklist Scores for adherence to a LFD and adherence to a MedDiet, at baseline and end intervention time points

4.7.9.2 Adherence to a Mediterranean Diet in the LFD and MedDiet group – the PREDIMED Score

Due to the overlap in dietary recommendations between both dietary intervention arms; the validated, 14-item PREDIMED score used to assess MedDiet adherence was assessed in both diet groups. Adherence to a MedDiet pattern using PREDIMED scores within each diet arm, from pre- to post-intervention, and then split into components of each checklist at pre- to post-intervention, are presented in **Figures 4.3, 4.4, and 4.5**, respectively.

Upon assessing MedDiet adherence in the LFD group, it was shown that participants significantly increased their PREDIMED scores from baseline to end-intervention (5.0 ± 1.8 to 6.4 ± 1.6, $p < 0.05$). In the LFD group, there was a significant increase in compliance for one diet component; preferentially choosing white meat over veal, pork, hamburger or sausage (pre-intervention compliance was 50% of participants, post-intervention compliance was 86% of participants, $p < 0.05$). Other diet components increased non-significantly, including; choosing EVOO as main culinary fat (23% to 33%, $p > 0.05$), consumption of ≥2 serves vegetables per day (59% to 76%, $p > 0.05$), consumption of <1 serve dairy fat per day (50% to 71%, $p > 0.05$) and ≥1 serve nuts per day (32% to 57%, $p > 0.05$). These changes were reflective of the overall cohort change.

As reported above, the MedDiet group significantly increased their PREDIMED scores from baseline to end-intervention (6.47 ± 1.98 to 9.17 ± 1.89, $p = 0.0005$). Individual components of the checklist that significantly increased were: choosing EVOO as the main culinary fat (63% of participants complying at baseline to 100% compliance at end-intervention, $p < 0.05$), consuming ≥4 tablespoons per day of EVOO (16% of participants complying at baseline to 44% compliance at end-intervention, $p < 0.05$), consumption of ≥3 serves fish per week (21% of participants complying at baseline to 83% complying at end intervention, $p < 0.05$), <3 serves of sweets per week (79% of participants complying at baseline to 56% compliance at end-intervention, $p < 0.05$), preferentially choosing white meat (58% of participants complying at baseline to 89% compliance at end-intervention, $p < 0.05$) and consumption of ≥2 serves per week food prepared using sofrito methods (32% of participants complying at baseline to 59% compliance at end-intervention, $p < 0.05$). The number of individual dietary component/recommendation improvements in the MedDiet group were greater than the changes seen in the LFD and overall study group.

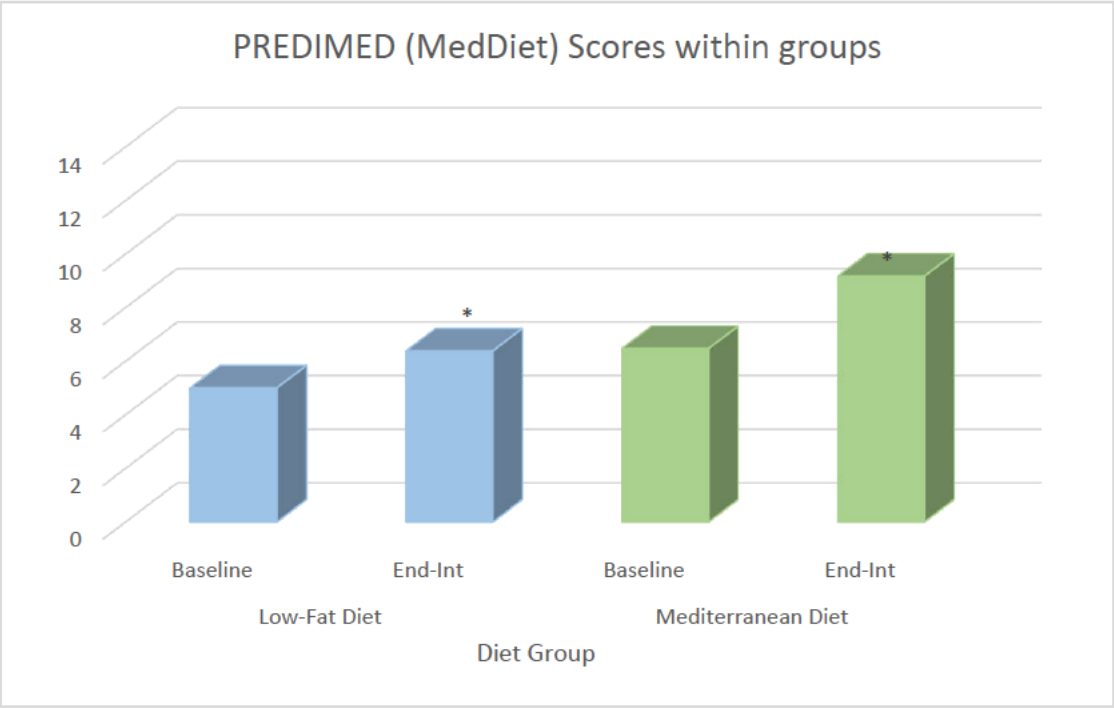


Figure 4.3. PREDIMED Scores for adherence to a Mediterranean Diet within the LFD and MedDiet groups at baseline and end-intervention

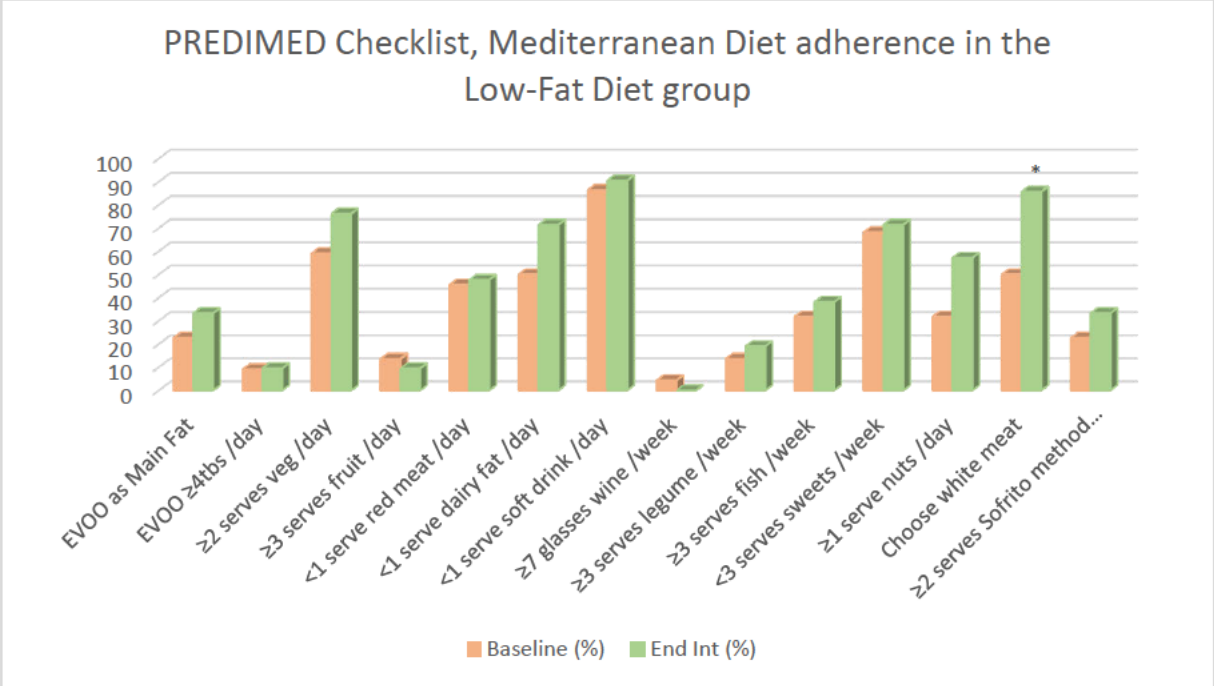


Figure 4.4. Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the LFD group at baseline and end-intervention

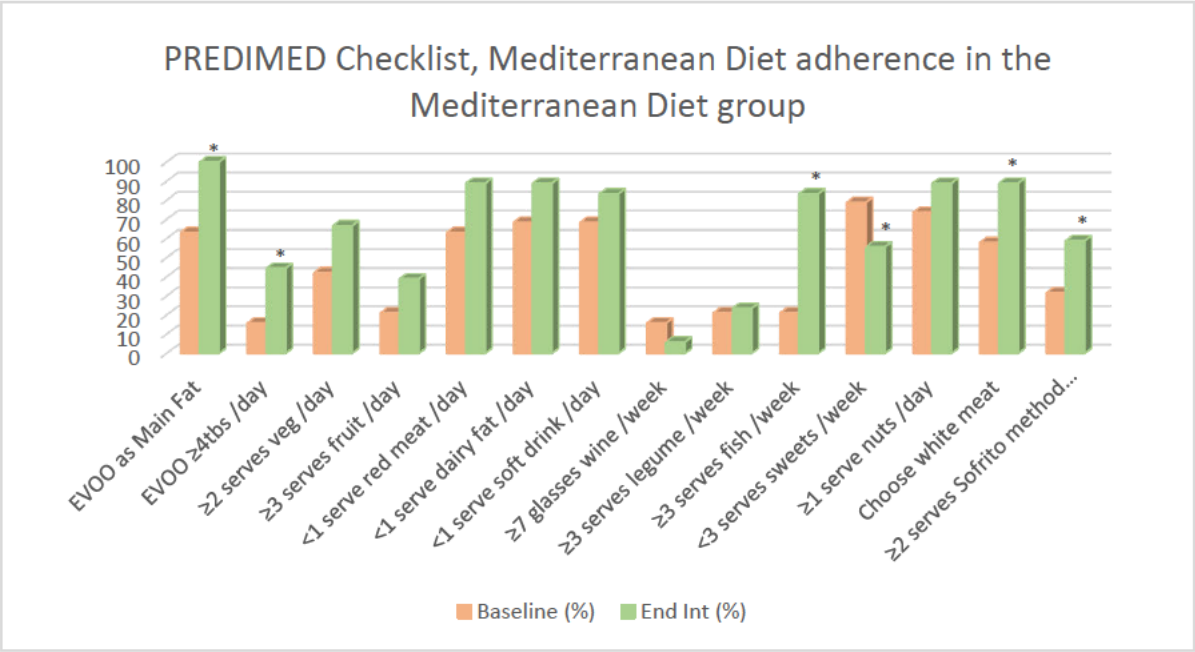


Figure 4.5. Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the MedDiet group at baseline and end-intervention.

4.7.9.3 The overall cohort's compliance to a Mediterranean Diet – The PREDIMED Score

In the total cohort (n=42), MedDiet adherence scores increased significantly from baseline to end-intervention (5.64 ± 2.02 to 7.67 ± 2.23, p =0.0005). These results are displayed in **Figure 4.6**.

Components of the checklist that significantly increased from baseline to end-intervention for the pooled/entire cohort include; choosing EVOO as the main culinary fat (41% to 64%, p =0.011), consuming ≥4 tablespoons per day of EVOO (12% to 26%, p =0.032), consumption of ≥2 serves vegetables per day (51% to 72%, p =0.031), consumption of <1 serve dairy fat per day (58% to 79%, p =0.044), consumption of ≥3 serves fish per week (27% to 59%, p =0.001), consumption of ≥1 serve nuts per day (51% to 72%, p =0.044) and preferentially choosing white meat over veal, pork, hamburger or sausage (54% to 87%, p =0.001). These results are displayed in **Figure 4.7**.

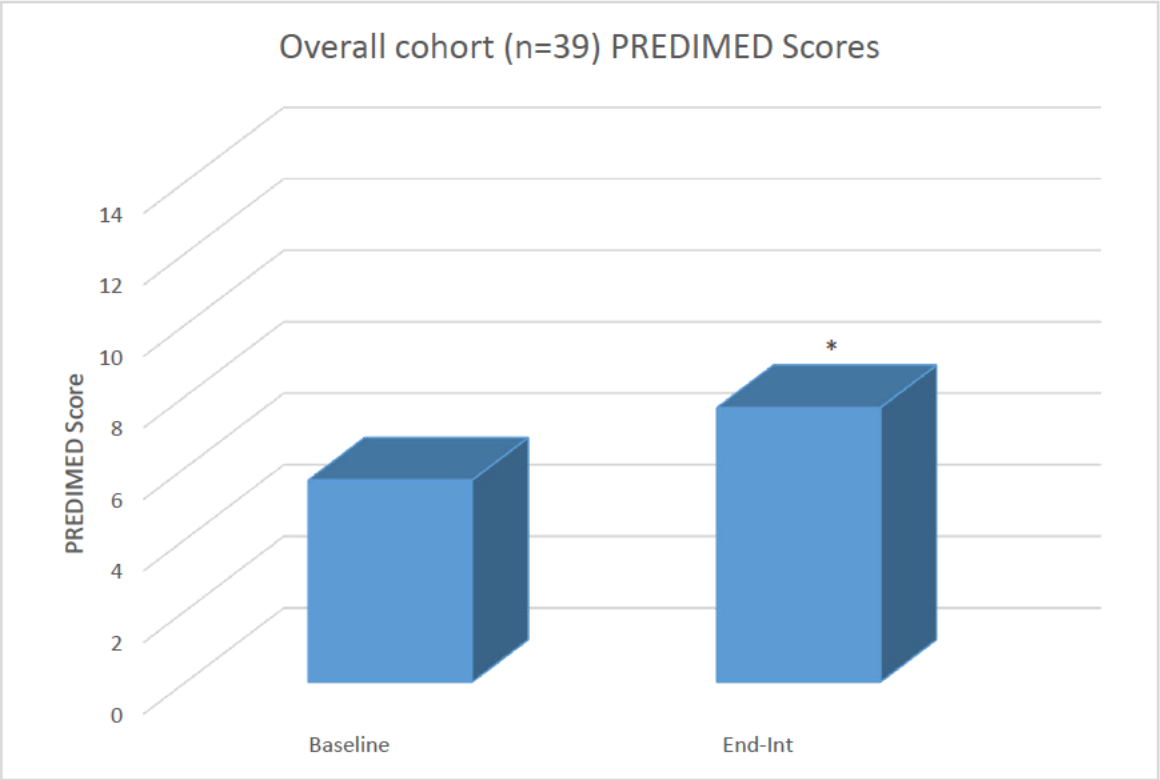


Figure 4.6. PREDIMED Scores of Mediterranean Diet adherence for the entire cohort of MEDINA participants at baseline and end-intervention

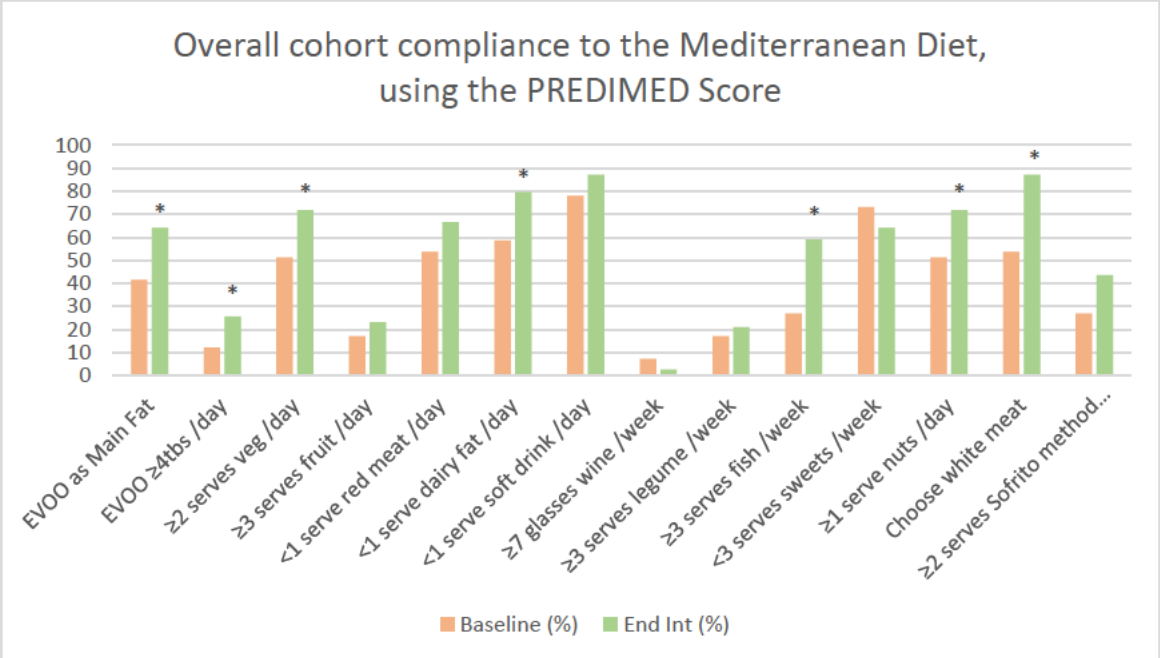


Figure 4.7. Individual components of the PREDIMED Checklist for Mediterranean Diet adherence in the overall NAFLD cohort at baseline and end-intervention.

4.7.10 Diet Quality in a NAFLD cohort – pooled analysis

Given that adherence to a MedDiet significantly improved in both of the dietary intervention groups, data for the entire MEDINA cohort was pooled to evaluate the difference in primary outcomes for participants who were more adherent to a MedDiet versus patients who were less adherent to a MedDiet (based on PREDIMED scores). From the PREDIMED checklist, key nutrients and food groups known to contribute to beneficial effects of a MedDiet were isolated and associations between these components and the primary outcomes of this study (inflammatory markers) were determined (**Table 4.10)**. Where significant associations existed, further (regression) analyses were conducted to determine the predictive potential of those variables on inflammatory markers following an overall improvement to diet quality. The aim of this analysis was to determine if there was an association between inflammatory markers, liver outcomes and IR with intake of key nutrient and food group components of a Mediterranean diet.

4.7.10.1 Difference in inflammatory markers, liver fat and stiffness measures, IR and anthropometry between higher and lower levels of adherence to a MedDiet (in the pooled cohort)

In order to explore the impact of a potential improvement in diet quality on inflammatory markers and key liver, metabolic and anthropometric measures for the entire MEDINA cohort, MedDiet (PREDIMED) scores were derived for the entire cohort and divided into lower versus higher MedDiet adherence (as displayed in **Table 4.9**). MedDiet adherence scores were split above and below the mean score of 7.66; below 7.65 was classified as low to moderate adherence to the MedDiet pattern and above 7.66 was classified as moderate to high adherence.

At the end-intervention timepoint (12 weeks), 18 participants were classified as having a low to moderate level of adherence to a MedDiet and 21 participants were classified as having a moderate to high level of adherence. As expected, (due to dietary prescriptions) within the lower dietary adherence group, 15 participants were in the LFD arm and three were in the MedDiet arm. Of those in the higher dietary adherence group, 15 participants were in the MedDiet arm and six were in the LFD arm.

As expected, the mean MedDiet score of the higher adherence group was significantly greater than the lower adherence group (9.3 ± 1.5 vs 5.7 ± 1.0, p =0.0005). The mean change in MedDiet score from baseline to end intervention was also significantly higher in the higher adherence group compared to lower adherence (+3.0 ± 1.8 vs +0.9 ± 1.8, p =0.001). At end-intervention circulating levels of inflammatory markers hs-CRP and leptin were significantly lower in individuals within the high adherence group compared to the low adherence group (1.7 ± 1.2mg/L vs 4.4 ± 2.7mg/L, p =0.002, and 11.2 ± 9.7ng/mL vs 20.1 ± 12.5ng/mL, p =0.017, respectively). While this trend was also seen for TNF-α and resistin, the difference between mean values for each group did not reach statistical significance (p >0.05).

IHL levels and LSM were lower in those with a higher MedDiet adherence, albeit not significantly (p >0.05). HOMA-IR was significantly lower in participants in the high MedDiet adherence group compared to the low MedDiet adherence group (3.2 ± 1.4 vs 6.6 ± 5.8, p =0.026), as were levels of fasting glucose (5.7 ± 1.2mmol/L vs 7.0 ± 2.5mmol/L, p =0.042) and insulin (12.8 ± 6.0mIU/L vs 19.7 ± 12.4mIU/L, p =0.043). There were no statistically significant differences in anthropometric or body composition measures; weight, BMI, FM or VF between levels of dietary adherence to a MedDiet.

Table 4.9. Mean values of inflammatory markers and key liver, metabolic and anthropometric measures for the entire MEDINA cohort at end-intervention, according to level of adherence to a MedDiet (PREDIMED scores)

	Adherence to a MedDiet (based on PREDIMED Scores)				
	Lower Adherence (4.00 - 7.65) n=18 LFD 15 / MD 3 T2DM 9 / Non-T2DM 9		**Higher Adherence (7.66 – 14.00)** n=21 LFD 6 / MD 15 T2DM 6 / Non-T2DM 15		
	mean	**SD**	**mean**	**SD**	**p-value**
PREDIMED Score	5.72	1.02	9.33	1.49	<0.001*
Change in MedDiet Score (From baseline to EI)	0.94	1.83	2.95	1.80	0.001*
hs-CRP (mg/L)	4.37	2.68	1.72	1.24	0.002*
TNF-α (pg/mL)	4.85	2.19	3.87	1.68	0.120
IL-6 (pg/mL)	10.68	14.57	16.45	24.08	0.381
Adiponectin (µg/mL)	18.62	21.42	18.14	13.77	0.933
Leptin (ng/mL)	20.07	12.45	11.19	9.74	0.017*
Resistin (ng/mL)	42.90	23.32	36.46	12.42	0.279
IHL (%)	12.73	12.55	8.79	8.52	0.306
LSM (kPa)	10.80	8.83	8.09	7.74	0.327
Glucose (mmol/L)	6.96	2.45	5.68	1.20	0.042*
Insulin (mIU/L)	19.67	12.41	12.81	6.00	0.043*
HOMA-IR	6.58	5.82	3.18	1.44	0.026*
Weight (kg)	87.03	18.65	87.71	21.92	0.918
BMI (kg/m^2)	31.83	5.21	31.30	5.21	0.755
Fat Mass (%)	40.59	6.98	37.42	8.13	0.203
Visceral Fat (L)	1.50	0.61	1.77	1.35	0.434

Abbreviations: MedDiet or MD, Mediterranean Diet; LFD, Low-Fat Diet; SD, standard deviation; EI, end-intervention; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IHL, intrahepatic lipid content; LSM, liver stiffness measure; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index. P-values compare differences between participants

in lower versus higher adherence groups; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. * indicates significance ($p < 0.05$).

4.7.10.2 Correlations between key nutrients and food groups known to contribute to the beneficial effects of a MedDiet and inflammatory markers (in the pooled cohort)

Given that diet quality improved in the overall MEDINA cohort from pre- to post-intervention (irrespective of assigned diet), a correlational analysis was undertaken to assess the linear association between food and nutrient components of a MedDiet pattern (isolated dietary intake components of the PREDIMED checklist) with circulating inflammatory markers. The aim of this analysis was to determine if associations exist between inflammatory markers and key nutrient and food group components of a Mediterranean-style dietary pattern. Partial correlation was used to assess this relationship and each model controlled for the baseline value of the corresponding inflammatory marker; all results are reported in **Table 4.10**.

hs-CRP was significantly ($p < 0.01$), moderately and positively correlated with consumption of protein and alcohol ($r = 0.50$ and 0.48, respectively). There were also significant ($p < 0.05$), moderate and negative correlations observed between hs-CRP with energy (kJ) intake, PREDIMED MedDiet score, PUFA and linoleic acid ($r = -0.44$, -0.39, -0.38 and -0.37, respectively).

IL-6 was significantly ($p < 0.05$), moderately and positively correlated with consumption of added sugars and SFA ($r = 0.49$ and 0.36) and moderately and negatively associated with intake of red meat ($r = -0.36$).

Leptin had a significant ($p < 0.05$), moderate and positive correlation with SFA ($r = 0.37$) and was negatively and moderately correlated with energy (kJ) intake, very long chain n-3 FAs, high long chain omega-3 (LCN3) seafood serves, ALA, linoleic acid and the PREDIMED MedDiet score ($r = -0.43$, -0.34, -0.38, -0.38, -0.35, -0.35 and -0.34).

There were no significant correlations observed between TNF-α, adiponectin or resistin with key nutrient and food group criteria of a MedDiet pattern.

Table 4.10. Partial correlations between inflammatory markers with nutrients and food groups in a Mediterranean-style dietary pattern (isolated dietary intake components of the PREDIMED checklist), with values adjusted for baseline inflammatory marker

	Correlation coefficient (Controlling for baseline value of inflammatory marker)					
	hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
PREDIMED MedDiet Score	-0.39*	-0.13	-0.07	0.04	-0.34*	-0.01
Energy (kJ)	-0.44*	0.11	-0.08	-0.23	-0.43**	-0.09
Protein (%E)	0.50**	-0.01	-0.09	0.24	-0.01	0.06
Carbohydrate (%E)	-0.10	-0.08	0.08	-0.14	-0.03	0.04
Added sugars (%E)	-0.28	-0.11	0.49**	-0.15	0.06	-0.01
Total fat (%E)	-0.32	0.13	-0.03	-0.01	0.02	-0.04
Saturated fat (%E)	-0.12	-0.03	0.36*	0.11	0.37*	0.06
Monounsaturated fat (%E)	-0.30	0.16	-0.18	-0.06	-0.13	-0.06
Polyunsaturated fat (%E)	-0.38*	0.21	-0.21	-0.08	-0.30	-0.07
Dietary fibre (g)	-0.31	0.05	-0.20	-0.16	-0.31	-0.10
Alcoholic drinks (serve)	0.29	-0.19	0.00	-0.09	-0.18	-0.15
Alcohol (%E)	0.48**	-0.18	0.09	-0.05	-0.05	-0.11
Caffeine (mg)	0.05	0.13	0.11	-0.07	0.17	-0.15
Wholegrains (serve)	-0.19	-0.22	-0.08	0.15	-0.12	-0.22
Linoleic acid (g)	-0.37*	0.16	-0.15	-0.17	-0.35*	-0.07
ALA (g)	-0.30	0.17	-0.16	-0.19	-0.35*	-0.02
VLC n-3 FA (g)	-0.18	0.17	0.00	0.09	-0.38*	-0.03
Vegetables (serve)	-0.27	0.19	-0.06	-0.25	-0.17	-0.26
Dark Green Vegetables (serve)	0.24	0.10	0.06	-0.30	-0.21	-0.06
Tomatoes (serve)	-0.18	-0.21	0.09	-0.03	-0.28	0.04
Legumes (serve)	-0.26	-0.05	-0.13	-0.17	0.05	0.09
Red meats (serve)	0.04	-0.05	0.36*	-0.05	-0.10	0.00
Poultry (serve)	0.13	0.08	-0.12	-0.11	-0.18	-0.07
Seafood High LCN3 (serve)	-0.22	0.12	0.07	0.06	-0.38*	-0.01
Nuts (serve)	-0.22	0.12	-0.09	-0.03	-0.10	0.04

4.7.11 Regression analysis: Components of a MedDiet pattern with inflammatory markers

The nutrients and food groups of a MedDiet that were significantly ($p < 0.05$) correlated with end-intervention inflammatory measures were assessed for their ability to predict inflammatory markers using hierarchical multiple (linear) regression analysis. Participant age, sex, diabetes status, diet group allocation, baseline body weight and the pre-intervention value of inflammatory marker were entered in a stepwise manner, and were controlled for in each regression analysis model to account for variations that could be influenced by these potentially confounding factors. Non-parametric variables were transformed using logarithm base 10 (log10).

hs-CRP

Multiple regression analysis was employed to assess the ability of protein (%E), PUFA (%E) and alcohol (%E) to predict end-intervention hs-CRP (log-transformed), after controlling for hs-CRP at baseline, age, body weight at baseline, sex, diabetes status and diet group allocation.

The total variance in hs-CRP explained by these variables was 68%, $R^2 = 0.68$, F (6, 24) = 6.83, $p < 0.001$. Step 1 of the model which adjusted for hs-CRP at baseline, age, body weight at baseline, sex, diabetes status and diet group accounted for 63% of the variance in hs-CRP, $R^2 = 0.63$, F (9, 21) = 4.96, $p = 0.001$. The addition of protein (%E), PUFA (%E) and alcohol (%E) to the regression model (step 2) accounted for an additional 5% variance in hs-CRP, $\Delta R^2 = 0.50$, ΔF (3, 21) = 1.08, $p = 0.379$.

Baseline level of hs-CRP was the strongest predictor of hs-CRP at end of intervention ($\beta = 0.63$, $p < 0.001$). Both PUFA (%E) and alcohol (%E) were significant predictors of hs-CRP, PUFA (%E) ($\beta = -0.35$, $p = 0.05$) and alcohol (%E) ($\beta = 0.27$, $p = 0.05$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for hs-CRP are reported in **Table 4.11**.

Table 4.11. Multiple linear regression model for components of a MedDiet pattern with outcome variable hs-CRP† at the end-intervention

Model				
hs-CRP†	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1 (Confounders)	Age	0.00 [-0.01, 0.01]	-0.03	0.85
	Sex	-0.06 [-0.34, 0.22]	-0.07	0.67
	Diet group	-0.04 [-0.24, 0.16]	-0.06	0.67
	Diabetes	-0.07 [-0.34, 0.20]	-0.09	0.60
	Weight (kg) at baseline	0.00 [-0.01, 0.00]	-0.09	0.53
	hs-CRP at baseline	0.79 [0.50, 1.07]	0.80	0.00*

Step 2	Age	0.00 [-0.01, 0.01]	-0.10	0.47
	Sex	0.06 [-0.24, 0.35]	0.07	0.70
	Diet group	0.07 [-0.13, 0.27]	0.09	0.49
	Diabetes	-0.16 [-0.41, 0.09]	-0.21	0.20
	Weight (kg) at	0.00 [-0.01, 0.00]	-0.05	0.71
	baseline	0.63 [0.36, 0.89]	0.63	0.00*
	hs-CRP at baseline	0.02 [-0.01, 0.04]	0.21	0.19
	Protein (%E)	-0.06 [-0.12, 0.00]	-0.35	0.05*
	PUFA (%E)	0.05 [0.00, 0.11]	0.27	0.05*
	Alcohol (%E)			

hs-CRP, high-sensitivity C-reactive protein; %E, percent of total energy intake. †Log (base 10)-transformed variable. *Significant, P <0.05.

IL-6

Multiple regression analysis was performed to assess the ability of added sugar (%E), SFA (%E) and red meat (serves) to predict end-intervention level of IL-6 (log-transformed), after controlling for IL-6 at baseline, age, body weight at baseline, sex, diabetes status and diet group.

The total variance explained by these variables was 83%, $R^2 = 0.83$, F (9, 28) = 15.35, $p < 0.001$. Step 1 of the model which adjusted for IL-6 at baseline, age, body weight at baseline, sex, diabetes status and diet group accounted for a significant 81% of the variance in IL-6, $R^2 = 0.81$, F (6, 31) = 23.23, $p < 0.001$. The addition of added sugar (%E), SFA (%E) and red meat (serves) to the regression model (step 2) accounted for an additional, non-significant 2% variance in IL-6, $\Delta R^2 = 0.02$, ΔF (3, 28) = 0.74, $p = 0.537$. Baseline level of IL-6 was the only significant predictor of IL-6 at end of intervention (β = 0.91, $p < 0.001$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for IL-6 are reported in **Table 4.12**.

Table 4.12. Multiple linear regression model for components of a MedDiet pattern with outcome variable IL-6† at end-intervention

Model				
IL-6†	**Predictor Variables**	***B* [95% CI]**	**β**	**P**
Step 1 (Confounders)	Age	-0.01 [-0.02, 0.00]	-0.12	0.17
	Sex	-0.05 [-0.34, 0.25]	-0.04	0.74
	Diet group	-0.03 [-0.23, 0.17]	-0.02	0.76
	Diabetes	-0.05 [-0.33, 0.23]	-0.04	0.72
	Weight (kg) at	0.00 [0.00, 0.00]	0.00	0.96
	baseline	0.97 [0.79, 1.15]	0.92	0.00*
	IL-6 at baseline			
Step 2	Age	-0.01 [-0.02, 0.00]	-0.15	0.12
	Sex	-0.09 [-0.42, 0.23]	-0.07	0.55
	Diet group	-0.04 [-0.25, 0.17]	-0.03	0.72
	Diabetes	-0.09 [-0.39, 0.20]	-0.07	0.53
	Weight (kg) at	0.00 [0.00, 0.01]	0.01	0.93
	baseline	0.96 [0.75, 1.17]	0.91	0.00*
	IL-6 at baseline	-0.12 [-0.33, 0.10]	-0.10	0.28
	Red Meat	0.02 [-0.02, 0.06]	0.09	0.41
	SFA (%E)	0.00 [0.00, 0.00]	-0.11	0.29
	Added Sugars (%E)			

IL-6, interleukin-6; SFA, saturated fatty acid; %E, percent of total energy intake. †Log (base 10)-transformed variable. *Significant, P <0.05.

Leptin

Multiple regression analysis was performed to assess the ability of SFA (%E), linoleic acid, ALA and VLC n-3 FA to predict end-intervention level of leptin (log-transformed), after controlling for leptin at baseline, age, body weight at baseline, sex, diabetes status and diet group.

The total variance explained by these variables was 89%, $R^2 = 0.89$, F (10, 27) = 21.87, $p < 0.001$. Step 1 of the model which adjusted for leptin at baseline, age, body weight at baseline, sex, diabetes status and diet group accounted for a significant 88% of the variance in leptin, $R^2 = 0.88$, F (6, 31) = 39.97, $p < 0.001$. The addition of SFA (%E), linoleic acid, ALA and VLC n-3 FA to the regression model (step 2) accounted for an additional, non-significant 1% variance in leptin, $\Delta R^2 = 0.01$, ΔF (4, 27) = 0.28, $p = 0.887$. Baseline level of leptin was the only significant predictor of leptin at end of intervention (β = 1.05, $p < 0.001$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for IL-6 are reported in **Table 4.13**.

Table 4.13. Multiple linear regression model for components of a MedDiet pattern with outcome variable leptin† at end-intervention

Model					
Leptin†	**Predictor Variables**	***B* [95% CI]**	**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	0.00 [-0.01, 0.00]	-0.04	0.63	
	Sex	0.08 [-0.11, 0.27]	0.11	0.39	
	Diet group	0.08 [-0.02, 0.18]	0.10	0.12	
	Diabetes	0.06 [-0.08, 0.21]	0.08	0.40	
	Weight (kg) at baseline	0.00 [-0.01, 0.00]	-0.10	0.29	
	Leptin at baseline	1.10 [0.85, 1.34]	0.96	0.00*	
Step 2	Age	0.00 [-0.01, 0.00]	-0.07	0.44	
	Sex	0.10 [-0.12, 0.31]	0.12	0.37	
	Diet group	0.07 [-0.05, 0.18]	0.09	0.24	
	Diabetes	0.06 [-0.10, 0.21]	0.07	0.47	
	Weight (kg) at baseline	0.00 [-0.01, 0.00]	-0.16	0.20	
	Leptin at baseline	1.20 [0.85, 1.54]	1.05	0.00*	
	SFA (%E)	0.00 [-0.02, 0.02]	-0.04	0.67	
	Linoleic Acid	0.00 [-0.01, 0.01]	0.08	0.46	
	ALA	-0.01 [-0.11, 0.10]	-0.01	0.89	
	VLC n-3 FA	0.03 [-0.04, 0.11]	0.09	0.40	

SFA, saturated fatty acid; %E, percent of total energy intake; ALA, alpha linoleic acid; VLC n-3 FA, very long chain omega-3 fatty acid. †Log (base 10)-transformed variable. *Significant, P <0.05.

4.7.11.1 Correlations between inflammatory markers, liver outcomes, biochemical, anthropometry and body composition variables (in the pooled cohort)

For the pooled cohort of patients with NAFLD, partial correlation was used to assess the linear relationship between circulating inflammatory markers, liver outcomes and measures of biochemistry, anthropometry and body composition, after controlling for weight change between baseline and end intervention.

4.7.11.2 Association between anthropometry, body composition and biochemistry with inflammatory markers (in the pooled cohort)

In order to assess the effect of the improvement in diet quality in the entire cohort, correlation coefficients for circulating inflammatory markers with biomarkers, anthropometry and body composition measures at the end-intervention timepoint were analysed and are presented in **Table 4.14**.

hs-CRP levels were moderately, positively and significantly ($p < 0.05$) correlated with BMI, NC, GGT, glucose, insulin and HOMA-IR ($r = 0.35$, 0.48, 0.38, 0.39, 0.41 and 0.49, respectively). Systolic blood pressure was moderately, inversely and significantly ($p < 0.05$) correlated with hs-CRP ($r = -0.38$).

TNF-α presented a strong, negative and significant ($p < 0.05$) correlation with HDL ($r = -0.52$), and a moderate, negative and significant ($p < 0.05$) correlation with cholesterol, LDL and GGT ($r = -0.41$, -0.38 and -0.39, respectively). Levels of serum IL-6 were significantly ($p < 0.05$) strongly and positively correlated with ALT and AST ($r = 0.51$ and $r = 0.55$, respectively), and had a moderate, positive and significant ($p < 0.05$) correlation with GGT ($r = 0.33$).

Adiponectin levels were significantly ($p < 0.05$), moderately and positively correlated with fat mass (kg), fat mass (%), fat mass index (kg/m^2) and HDL ($r = 0.38$, 0.47, 0.42 and 0.42, respectively). Circulating levels of leptin showed strong, positive significant ($p < 0.05$) correlations with fat mass (%) and fat mass index (kg/m^2) ($r = 0.52$ and 0.50, respectively) and moderate, positive correlations with HC (cm) and fat mass (kg) ($r = 0.45$ and 0.38, respectively). There was a significant ($p < 0.05$), moderate and negative correlation between leptin and levels of albumin ($r = -0.42$). Resistin was significantly ($p < 0.05$), strongly and positively correlated with NC (cm) ($r = 0.61$), and moderately and negatively correlated with systolic and diastolic blood pressure ($r = -0.43$ and -0.35, respectively).

Table 4.14. Partial correlations between inflammatory markers, anthropometric and biochemical variables at end intervention, values adjusted for mean weight-loss

	Correlation Coefficients (adjusted for weight loss)					
	hs-CRP	TNF-alpha	IL-6	Adiponectin	Leptin	Resistin

Liver outcomes and biochemistry						
IHL (%)	0.20	0.27	0.08	-0.04	0.19	-0.09
LSM (kPa)	0.42*	0.12	0.07	0.01	0.18	0.39*
Glucose (mmol/L)	0.39*	0.24	0.06	-0.28	-0.09	0.12
Insulin (mIU/L)	0.41*	0.25	0.01	-0.12	0.30	0.27
HOMA-IR	0.49**	0.30	0.03	-0.23	0.23	0.26
ALT (U/L)	-0.06	0.01	0.51**	-0.02	0.02	0.05
AST (U/L)	0.01	0.08	0.55**	-0.06	-0.06	0.03
GGT (U/L)	0.38*	-0.39*	0.33*	0.17	0.09	0.31
ALP (U/L)	0.27	0.06	-0.08	0.30	0.03	0.19
Cholesterol (mmol/L)	-0.11	-0.41*	0.19	0.19	0.24	-0.12
HDL (mmol/L)	-0.04	-0.52**	0.18	0.42**	0.32	0.09
LDL (mmol/L)	-0.14	-0.39*	0.10	0.16	0.17	-0.14
Triglycerides (mmol/L)	0.07	0.12	0.32	-0.11	0.11	-0.06
Anthropometry, Body Composition and Haemodynamic Measures						
Weight (kg)	0.22	0.20	-0.11	0.19	0.15	0.19
BMI (kg/m^2)	0.35*	0.06	-0.09	0.26	0.32	0.13
WC (cm)	0.26	0.12	-0.10	0.18	0.31	0.16
HC (cm)	0.21	0.18	-0.16	0.29	0.45**	0.21
WHR	0.15	-0.15	0.12	-0.23	-0.30	-0.06
NC (cm)	0.48**	0.13	0.03	-0.19	-0.07	0.61**
Fat Mass (kg)	0.24	0.08	-0.16	0.38*	0.38*	0.10
Fat Mass (%)	0.16	-0.09	-0.14	0.47**	0.52**	-0.01
Fat Mass Index (kg/m^2)	0.31	-0.03	-0.13	0.42**	0.50**	0.09
Blood Pressure (Systolic)	-0.38*	-0.12	0.02	-0.02	-0.15	-0.43**
Blood Pressure (Diastolic)	-0.32	-0.09	0.02	0.13	0.14	-0.35*

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.
hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

4.7.11.3 Association between nutrient and food group intake with inflammatory markers (in the pooled cohort)

Correlation coefficients for primary inflammatory outcomes and nutrient and dietary food group intake, at the end-intervention timepoint are presented in **Table 4.15**. Additional, non-significant variables are presented in **Appendix 11; Supplementary Table 5**.

Circulating levels of hs-CRP were significantly ($p < 0.05$) moderately and negatively correlated with energy (kJ), carbohydrates (g), sugars (g), total fat (g), MUFA (g), PUFA (g), PUFA (%E), linoleic acid, vitamin E and serves of oil equivalents (r =-0.44, -0.45, -0.38, -0.39, -0.36, -0.39, -0.38, -0.37,

-0.36 and -0.39, respectively). Hs-CRP was also significantly ($p < 0.05$), moderately and negatively correlated with PREDIMED scores for adherence to a Mediterranean Diet ($r = -0.39$).

No correlation between TNF-α and dietary intake variables was found. Adherence to a LFD as determined by PREDIMED checklist scores showed a significant ($p < 0.05$), moderate and positive correlation with TNF-α ($r = 0.48$). Serum IL-6 levels were significantly ($p < 0.05$) moderately and positively associated with sugar (%E), saturated fat (%E) and saturated fat (% total fat), serves of dairy, serves of milk and added sugars ($r = 0.39$, 0.36, 0.47, 0.37, 0.47 and 0.49, respectively). IL-6 was also significantly ($p < 0.05$), moderately and negatively associated with MUFA (% total fat) and red meats ($r = -0.38$ and -0.36).

There were many significant associations found between circulating levels of leptin and dietary intake variables. Moderate and positive significant correlations were observed for leptin and SFA (%E), SFA (as % total fat) and serves of processed meats ($r = 0.37$, 0.50 and 0.38, respectively). Significant ($p < 0.05$), moderate and negative correlations were observed between leptin and energy (kJ), protein (g), carbohydrate (g), MUFA (g), PUFA (g), PUFA (% total fat), tocopherol alpha, vitamin E, potassium, magnesium, iron and zinc ($r = -0.43$, -0.45, -0.41, -0.35, -0.41, -0.43, -0.36, -0.34, -0.42, -0.35, -0.36 and -0.42, respectively). Leptin was also significantly ($p < 0.05$) moderately and negatively associated with very long chain omega 3 fatty acids ($r = -0.38$), including all components; linoleic acid, ALA, EPA, DPA and DHA ($r = -0.35$, -0.35, -0.36, -0.35 and -0.38, respectively). Components of food group intake that were significant ($p < 0.05$) and moderately and negatively associated with leptin were grains ($r = -0.39$), specifically refined grains (-0.35), seafood with high long chain omega-3 content ($r = -0.38$) and servings of oil equivalents ($r = -0.42$). Finally, leptin was significant ($p < 0.05$) and inversely correlated with PREDIMED scores for adherence to a Mediterranean Diet ($r = -0.34$). There were no significant associations between adiponectin or resistin and dietary intake variables.

Table 4.15. Partial correlations between inflammatory markers, nutrient and dietary food group variables at end intervention, values adjusted for mean weight-loss

	Correlation Coefficients (adjusted for weight loss)					
	hs-CRP	TNF-alpha	IL-6	Adiponectin	Leptin	Resistin
Macronutrients						
Energy (kJ)[b]	-.435*	0.11	-0.08	-0.23	-.429**	-0.09
Protein (g)[a]	-0.28	0.14	-0.18	-0.10	-.448**	-0.05
Protein (% of total E)	.497**	-0.01	-0.09	0.24	-0.01	0.06
Carbohydrate (g)[a]	-.449**	0.05	-0.02	-0.25	-.408*	-0.07
Sugars (g)[b]	-.384*	0.03	0.29	-0.29	-0.13	0.02
Sugars (% of total E)	0.01	-0.02	.386*	-0.21	0.28	0.10
Total fat (g)[b]	-.395*	0.13	-0.04	-0.20	-0.32	-0.08
Saturated fat (% of total E)	-0.12	-0.03	.364*	0.11	.374*	0.06

Saturated fat (% of total fat)	0.29	-0.11	.466**	0.19	.500**	0.18
Mono-unsaturated Fat (g)[b]	-.366*	0.16	-0.13	-0.22	-.345*	-0.08
Mono-unsaturated fat (% of total E)	-0.30	0.16	-0.18	-0.06	-0.13	-0.06
Mono-unsaturated fat (% of total fat)	-0.17	0.09	-.376*	-0.12	-0.29	-0.11
Poly-unsaturated Fat (g)[b]	-.389*	0.19	-0.153	-0.16	-.406*	-0.07
Poly-unsaturated fat (% of total E)	-.376*	0.21	-0.21	-0.08	-0.30	-0.07
Poly-unsaturated fat (% of total fat)	-0.24	0.07	-0.28	-0.16	-.425**	-0.15
Tocopherol Alpha[b]	-0.35	0.25	-0.18	-0.15	-.366*	-0.09
Linoleic Acid[b]	-.368*	0.16	-0.15	-0.17	-.348*	-0.07
Alpha Linolenic Acid (ALA)[b]	-0.30	0.17	-0.16	-0.19	-.354*	-0.02
Eicosapentaenoic acid (EPA)[b]	-0.14	0.19	-0.06	0.14	-.358*	0.00
Docosapentaenoic acid (DPA)[b]	-0.04	0.17	-0.10	0.14	-.346*	0.00
Docosahexaenoic acid (DHA)[b]	-0.23	0.13	0.08	0.04	-.380*	-0.07
Very Long Chain	-0.18	0.17	0.00	0.09	-.383*	-0.03
Vitamin E[b]	-.361*	0.22	-0.10	-0.16	-.342*	-0.07
Potassium[a]	-0.31	0.13	-0.11	-0.16	-.417**	-0.13
Magnesium[a]	-0.33	0.14	-0.12	-0.14	-.346*	0.00
Iron[b]	-0.32	0.11	-0.04	-0.10	-.361*	0.02
Zinc[a]	-0.22	0.11	-0.11	-0.15	-.424**	0.05
Food Groups						
Grains[b]	-0.29	0.04	-0.15	-0.10	-.398*	-0.07
Refined Grains[b]	-0.20	0.16	-0.11	-0.18	-.350*	0.04
Red meats[b]	0.04	-0.05	-.356*	-0.05	-0.10	0.00
Processed meats[b]	-0.07	0.01	0.22	0.02	.380*	0.10
Seafood						
High long chain omega-3[b]	-0.22	0.12	0.07	0.06	-.380*	-0.01
Dairy[b]	-0.08	-0.03	.376*	0.16	0.01	-0.06
Milk	0.04	0.05	.473**	-0.11	0.04	-0.06
Oil Equivalents[b]	-.397*	0.19	-0.21	-0.18	-.419**	-0.16
Added Sugars[b]	-0.28	-0.11	.493**	-0.15	0.06	-0.01
Adherence to a LFD (PREDIMED Score)	0.27	.484*	0.22	0.09	-0.08	-0.05
Adherence to a MedDiet (PREDIMED Score)	-.388*	-0.13	-0.07	0.04	-.343*	-0.01

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.7.12 Regression analysis: liver outcomes, biochemistry, anthropometry, and body composition with inflammatory markers

In order to test the predictive ability of the anthropometric, biochemical, and dietary intake variables significant in the correlational analysis for each cytokine and adipokine marker, multiple (linear) regression analyses were performed. Participant age, sex, diabetes status, pre-intervention body weight and study group allocation were controlled for in each regression model to account for variations that could be influenced by these potentially confounding factors. Non-parametric

variables were transformed using logarithm base 10 (log10). The inflammatory marker resistin remained non-parametric after being transformed using log10, therefore it was transformed using Square Root (sqrt) transformation.

hs-CRP

Multiple regression analysis was employed at the end-intervention timepoint to assess the ability of BMI, insulin resistance, HOMA-IR and resistin (sqrt-transformed) to predict hs-CRP (log-transformed), after controlling for age, baseline body weight, sex, diabetes status and diet group.

On step 1 of the hierarchical model, adjustment for age, baseline body weight, sex, diabetes status and diet group accounted for 14% of the variance in hs-CRP, $R^2 = 0.14$, $F\ (5, 27) = 0.893$, $p = 0.500$. On step 2, the addition of BMI to the model accounted for an additional 8% variance in hs-CRP, $\Delta R^2 = 0.08$, $\Delta F\ (1, 26) = 2.77$, $p = 0.108$. On step 3, the addition of HOMA-IR to the regression model accounted for an additional and significant 16% variance in hs-CRP, $\Delta R^2 = 0.15$, $\Delta F\ (1, 25) = 6.17$, $p = 0.020$. At step 4, the addition of resistin accounted for an additional, significant 10% variance in hs-CRP, $\Delta R^2 = 0.010$, $\Delta F\ (1, 24) = 4.23$, $p = 0.044$. In combination, the total variance explained by the three predictor variables and confounders was 48%, $R^2 = 0.48$, $F\ (8, 24) = 2.73$, $p = 0.027$. Both HOMA-IR and resistin were significant predictors of hs-CRP, though HOMA-IR ($\beta = 0.372$, $p = 0.035$) recorded a higher beta than resistin ($\beta = 0.358$, $p = 0.044$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for hs-CRP are reported in **Table 4.16**.

Table 4.16. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable hs-CRP† at the end-intervention

Model					
hs-CRP†	**Predictor Variables**	***B* [95% CI]**	**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	0.00 [-0.01, 0.02]	0.10	0.62	0.01
	Weight (kg) at baseline	0.00 [0.00, 0.01]	0.20	0.32	0.03
	Sex	0.10 [-0.30, 0.50]	0.13	0.61	0.01
	Diabetes	-0.06 [-0.46, 0.33]	-0.08	0.74	0.00
	Diet group	-0.18 [-0.46, 0.11]	-0.23	0.21	0.05
Step 2	Age	0.00 [-0.01, 0.01]	0.04	0.84	0.00
	Weight (kg) at baseline	0.00 [-0.02, 0.01]	-0.25	0.45	0.02
	Sex	0.11 [-0.28, 0.49]	0.14	0.57	0.01
	Diabetes	-0.06 [-0.44, 0.33]	-0.07	0.76	0.00
	Diet group	-0.22 [-0.50, 0.06]	-0.28	0.13	0.07
	BMI	0.04 [-0.01, 0.09]	0.53	0.11	0.08
Step 3	Age	0.00 [-0.01, 0.01]	0.09	0.63	0.01
	Weight (kg) at baseline	0.00 [-0.01, 0.01]	-0.19	0.53	0.01
	Sex	0.11 [-0.24, 0.46]	0.14	0.52	0.01
	Diabetes	0.06 [-0.31, 0.42]	0.07	0.75	0.00
	Diet group	-0.19 [-0.44, 0.07]	-0.24	0.15	0.05
	BMI	0.03 [-0.02, 0.07]	0.36	0.24	0.04
	HOMA-IR	0.56 [0.10, 1.03]	0.44	0.020*	0.15

Step 4	Age	0.00 [-0.01, 0.01]	0.04	0.83	0.00
	Weight (kg) at	-0.01 [-0.02, 0.00]	-0.43	0.17	0.04
	baseline	0.09 [-0.24, 0.42]	0.12	0.57	0.01
	Sex	0.10 [-0.25, 0.44]	0.12	0.57	0.01
	Diabetes	-0.23 [-0.48, 0.02]	-0.30	0.07	0.08
	Diet group	0.04 [-0.01, 0.08]	0.52	0.08	0.07
	BMI	0.48 [0.04, 0.93]	0.37	0.035*	0.11
	HOMA-IR	0.09 [0.00, 0.19]	0.36	0.044*	0.10
	Resistin ǂ				

hs-CRP, high-sensitivity C-reactive protein; BMI, Body Mass Index; HOMA-IR, homeostatic model assessment-insulin resistance. †Log (base 10)-transformed variable. ǂ Reciprocal transformed variable. *Significant, P <0.05.

TNF-α

Hierarchical multiple regression analysis was used to assess the assess the ability of one biochemical variable (HDL) to predict post-intervention levels of TNF-α, after controlling for age, baseline body weight, sex, diabetes status and diet group.

Age, baseline body weight, sex, diabetes status and diet group were first adjusted for (step 1) accounting for a non-significant 9% of the variance in TNF-α, $R^2 = 0.09$, $F(5, 33) = 0.63$, $p = 0.676$. After the addition of HDL to the regression model (step 2), an additional, significant 36% of the variance in TNF-α was accounted for, $\Delta R^2 = 0.36$, $\Delta F(1, 32) = 21.17$, $p = 0.0005$. The total variance explained by the final model was 45%, , $R^2 = 0.45$, $F(6, 32) = 4.38$, $p = 0.002$. In the final model, both age and HDL were significant predictors of TNF-α, with HDL recording a higher beta value (β = -.747, $p = 0.0005$) than age (β = 0.336, $p = 0.049$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for TNF-α are reported in **Table 4.17**.

Table 4.17. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable TNF-α at the end-intervention

Model					
TNF-α	**Predictor Variables**	***B* [95% CI]**	**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	0.01 [-0.05, 0.07]	0.05	0.79	0.00
	Weight (kg) at baseline	0.01 [-0.02, 0.04]	0.14	0.44	0.02
	Sex	-0.73 [-2.57, 1.12]	-0.18	0.43	0.02
	Diabetes	-0.95 [-2.80, 0.90]	-0.24	0.30	0.03
	Diet group	-0.32 [-1.65, 1.02]	-0.08	0.63	0.01
Step 2	Age	0.05 [0.00, 0.10]	0.34	0.049*	0.07
	Weight (kg) at	0.02 [-0.01, 0.05]	0.24	0.12	0.04
	baseline	1.07 [-0.59, 2.73]	0.27	0.20	0.03
	Sex	0.59 [-1.02, 2.20]	0.15	0.46	0.01
	Diabetes	-0.37 [-1.43, 0.68]	-0.10	0.47	0.01
	Diet group	-14.16 [-20.43, -7.89]	-0.75	<0.001*	0.36
	HDL				

TNF-α, tumor necrosis factor-alpha; HDL, high-density lipoprotein. *Significant, P <0.05.

IL-6

A hierarchical multiple regression analysis was performed to assess the ability of one biochemical variable (ALT) and one dietary food group variable (serves of Added Sugars) to predict post-intervention levels of IL-6 (log-transformed), after controlling for age, baseline body weight, sex, diabetes status and diet group.

Age, baseline body weight, sex, diabetes status and diet group were first controlled for within this model (step 1) which accounted for a non-significant 8% of the variance in IL-6, $R^2 = 0.08$, F (5, 33) = 0.62, $p = 0.689$. On step 2 of the multiple regression model, ALT accounted for an additional significant 8% variance in IL-6, $\Delta R^2 = 0.08$, ΔF (1, 32) = 2.89, $p = 0.098$. On step 3, dietary variable added sugars was then added to the model which accounted for an additional, significant 11% of the variance in IL-6, $\Delta R^2 = 0.11$, ΔF (1, 31) = 4.55, $p = 0.041$. The total variance explained by the final model was 27%, , $R^2 = 0.27$, F (7, 31) = 1.63, $p = 0.165$, with added sugar the only significant predictor in the model (β = 0.373, $p = 0.041$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for IL-6 are reported in **Table 4.18**.

Table 4.18. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable IL-6† at the end-intervention

Model							
IL-6†	**Predictor Variables**	***B* [95% CI]**			**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	-0.01	-0.03	0.01	-0.12	0.54	0.01
	Weight (kg) at baseline	0.00	-0.01	0.01	0.04	0.82	0.00
	Sex	0.46	-0.14	1.07	0.36	0.13	0.07
	Diabetes	0.08	-0.52	0.68	0.06	0.79	0.00
	Diet group	0.02	-0.41	0.46	0.02	0.91	0.00
Step 2	Age	0.00	-0.02	0.02	-0.03	0.89	0.00
	Weight (kg) at baseline	0.00	-0.01	0.01	0.01	0.97	0.00
	Sex	0.25	-0.40	0.89	0.19	0.44	0.02
	Diabetes	-0.12	-0.76	0.52	-0.09	0.70	0.00
	Diet group	-0.06	-0.50	0.38	-0.05	0.77	0.00
	ALT	0.89	-0.18	1.96	0.33	0.10	0.08
Step 3	Age	0.00	-0.02	0.02	0.07	0.73	0.00
	Weight (kg) at baseline	0.00	-0.01	0.01	0.01	0.96	0.00
	Sex	0.14	-0.48	0.76	0.11	0.65	0.01
	Diabetes	-0.20	-0.81	0.41	-0.15	0.51	0.01
	Diet group	-0.13	-0.56	0.29	-0.11	0.52	0.01
	ALT	1.00	-0.02	2.02	0.37	0.054	0.09
	Added Sugars	0.05	0.00	0.09	0.35	0.041*	0.11

IL-6, interleukin-6; ALT, alanine transaminase. †Log (base 10)-transformed variable. *Significant, P <0.05.

Adiponectin

Hierarchical multiple regression analysis was utilised to assess the ability of one anthropometric variable (FM (%)) and one biochemical variable (HDL) to predict post-intervention levels of

adiponectin (log-transformed), after controlling for age, baseline body weight, sex, diabetes status and diet group.

On step 1, controlling for age, baseline body weight, sex, diabetes status and diet group accounted for a significant 39% of the variance in adiponectin, $R^2 = 0.39$, F (5, 33) = 4.24, $p = 0.004$. On step 2, the addition of FM (%) to the regression equation accounted for an added, non-significant 1.4% of the variance in adiponectin, $\Delta R^2 = 0.014$, ΔF (1, 32) = 0.77, $p = 0.386$. On step 3, HDL was added to the model which accounted for an additional 0.3% variation of adiponectin, $\Delta R^2 = 0.003$, ΔF (1, 31) = 0.17, $p = 0.686$. In combination, the total variance explained by this regression model for adiponectin was 41%, $R^2 = 0.41$, F (7, 31) = 3.06, $p = 0.014$, and there were two statistically significant predictors of adiponectin. Baseline body weight recorded a higher beta value (β = 0.545, $p = 0.002$) than age (β = 0.353, $p = 0.050$). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for adiponectin are reported in **Table 4.19**.

Table 4.19. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Adiponectin† at the end-intervention

Model					
Adiponectin†	**Predictor Variables**	***B* [95% CI]**	**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	0.01 [0.00, 0.02]	0.40	0.015*	0.12
	Weight (kg) at baseline	0.01 [0.01, 0.02]	0.60	<0.001*	0.29
	Sex	0.23 [-0.09, 0.55]	0.27	0.15	0.04
	Diabetes	0.15 [-0.17, 0.47]	0.18	0.34	0.02
	Diet group	0.04 [-0.20, 0.27]	0.04	0.76	0.00
Step 2	Age	0.01 [0.00, 0.02]	0.38	0.026*	0.10
	Weight (kg) at baseline	0.01 [0.00, 0.02]	0.54	0.002*	0.21
	Sex	0.17 [-0.19, 0.52]	0.20	0.34	0.02
	Diabetes	0.15 [-0.17, 0.47]	0.18	0.35	0.02
	Diet group	0.03 [-0.20, 0.26]	0.04	0.78	0.00
	FM (%)	0.01 [-0.01, 0.03]	0.15	0.39	0.01
Step 3	Age	0.01 [0.00, 0.02]	0.35	0.050*	0.08
	Weight (kg) at baseline	0.01 [0.00, 0.02]	0.54	0.002*	0.21
	Sex	0.14 [-0.24, 0.52]	0.17	0.45	0.01
	Diabetes	0.12 [-0.25, 0.48]	0.14	0.52	0.01
	Diet group	0.03 [-0.20, 0.27]	0.04	0.77	0.00
	FM (%)	0.01 [-0.01, 0.03]	0.11	0.56	0.01
	HDL	0.31 [-1.25, 1.88]	0.08	0.69	0.00

FM (%), Fat Mass (percent); HDL, high-density lipoprotein. †Log (base 10)-transformed variable.
*Significant, P <0.05.

Leptin

Hierarchical multiple regression analysis was employed to assess the ability of one anthropometric variable (FM (%)) and one dietary intake variable (saturated fat (as % total fat)) to predict post-intervention levels of leptin (log-transformed), after controlling for age, baseline body weight, sex, diabetes status and diet group.

On step 1 of the hierarchical regression model, age, baseline body weight, sex, diabetes status and diet group were controlled for which accounted for a significant 57% of the variance in leptin, R^2 = 0.57, F (5, 32) = 8.57, p = 0.0005. On step 2, FM (%) was added to the regression equation and accounted for an additional significant 2% of the variance in leptin, ΔR^2= 0.02, ΔF (1, 31) = 1.66, p = 0.207. On step 3, the diet variable saturated fat (% of total fat) was added to the regression model and added an additional, significant 5% variance in leptin, ΔR^2= 0.05, ΔF (1, 30) = 4.47, p = 0.043. In combination, the two predictor variables and confounders explained a total variance of 64% in leptin, R^2 = 0.64, F (7, 30) = 7.85, p = 0.0005, with four variables found to be statistically significant predicators of leptin. The highest beta value was for sex (β = 0.70, p = 0.0005) then baseline body weight (β = 0.47, p = 0.001), diabetes (β = 0.45, p = 0.005) and saturated fat (% of total fat) (β = 0.26, p = 0.043). Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations *(sr^2)* for each predictor on each step of the hierarchical multiple regression model for leptin are reported in **Table 4.20**.

Table 4.20. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Leptin at the end-intervention

Model					
Leptin	**Predictor Variables**	***B* [95% CI]**	**β**	**P**	***sr^2***
Step 1 (Confounders)	Age	0.01 [0.00, 0.01]	0.18	0.18	0.02
	Weight (kg) at baseline	0.01 [0.00, 0.01]	0.53	<0.001*	0.23
	Sex	0.70 [0.45, 0.95]	0.91	<0.001*	0.43
	Diabetes	0.36 [0.11, 0.61]	0.47	0.006*	0.11
	Diet group	-0.09 [-0.27, 0.09]	-0.12	0.32	0.01
Step 2	Age	0.00 [0.00, 0.01]	0.15	0.28	0.02
	Weight (kg) at baseline	0.01 [0.00, 0.01]	0.46	0.002*	0.15
	Sex	0.63 [0.36, 0.90]	0.82	<0.001*	0.29
	Diabetes	0.36 [0.11, 0.60]	0.47	0.006*	0.11
	Diet group	-0.09 [-0.27, 0.09]	-0.12	0.30	0.01
	FM (%)	0.01 [-0.01, 0.02]	0.18	0.21	0.02
Step 3	Age	0.01 [0.00, 0.01]	0.17	0.19	0.02
	Weight (kg) at baseline	0.01 [0.00, 0.01]	0.47	0.001*	0.16
	Sex	0.54 [0.26, 0.81]	0.70	<0.001*	0.19
	Diabetes	0.34 [0.11, 0.58]	0.45	0.005*	0.11
	Diet group	-0.03 [-0.21, 0.15]	-0.04	0.71	0.00
	FM (%)	0.01 [0.00, 0.02]	0.19	0.17	0.02
	Saturated Fat (% of total fat)	0.01 [0.00, 0.03]	0.26	0.043*	0.05

FM (%), Fat Mass (percent). †Log (base 10)-transformed variable. *Significant, P <0.05.

Resistin

A hierarchical multiple regression analysis was performed to assess the ability of inflammatory marker hs-CRP (log-transformed) to predict post-intervention levels of resistin (sqrt-transformed), after controlling for age, baseline body weight, sex, diabetes status and diet group.

On step 1 of the regression model, age, baseline body weight, sex, diabetes status and diet group accounted for 16% variance for resistin, $R^2 = 0.16$, F (5, 27) = 1.04, $p = 0.414$. On step 2, hs-CRP was added to the regression equation, which accounted for an additional, non-significant 9% variance in resistin, $\Delta R^2 = 0.09$, ΔF (1, 26) = 3.16, $p = 0.087$. Overall, the whole regression model accounted for a non, significant 25% variance in resistin, $R^2 = 0.25$, F (6, 26) = 1.46, $p = 0.229$, and there were no significant predictor variables in this model. Unstandardised *(B)* and standardised (β) regression coefficients and squared semi-partial correlations (sr^2) for each predictor on each step of the hierarchical multiple regression model for resistin are reported in **Table 4.21**.

Table 4.21. Multiple linear regression model for liver outcomes, biochemistry, anthropometry and body composition with outcome variable Resistin ǂ at the end-intervention

Model							
Resistin ǂ	**Predictor Variables**	***B* [95% CI]**			**β**	**P**	sr^2
Step 1 (Confounders)	Age	0.01	-0.04	0.06	0.08	0.70	0.00
	Weight (kg) at baseline	0.02	-0.01	0.05	0.32	0.11	0.08
	Sex	0.22	-1.27	1.71	0.07	0.76	0.00
	Diabetes	-0.56	-2.05	0.92	-0.19	0.44	0.02
	Diet group	0.31	-0.76	1.39	0.11	0.55	0.01
Step 2	Age	0.01	-0.04	0.05	0.04	0.82	0.00
	Weight (kg) at baseline	0.02	-0.01	0.04	0.25	0.19	0.05
	Sex	0.10	-1.35	1.54	0.03	0.89	0.00
	Diabetes	-0.48	-1.92	0.95	-0.16	0.49	0.01
	Diet group	0.54	-0.53	1.60	0.18	0.31	0.03
	hs-CRP†	1.24	-0.19	2.67	0.33	0.09	0.09

hs-CRP, high-sensitivity C-reactive protein. †Log (base 10)-transformed variable. ǂ Reciprocal transformed variable. *Significant, P <0.05.

4.8 Section Two – Assessing the impact of a dietary intervention and overall improved diet quality in patients diagnosed with NAFLD and T2DM – Subgroup Analysis

4.8.1 The effect of a LFD vs a MedDiet within the NAFLD Type 2 Diabetes Cohort

Given there were differences in the representation of individuals with diabetes across dietary intervention groups, further analysis was conducted in the subset of the population diagnosed with T2DM versus those without T2DM in order to assess their responsiveness to diet. The following analysis explored the effect of the LFD and MedDiet on inflammatory markers, liver outcomes and biochemical and anthropometric measures in those with and without T2DM. The mean values of variables at pre-and post-intervention timepoints are presented in **Table 4.22**.

4.8.2 Population Characteristics

In this MEDINA cohort (n=42), there were 18 individuals diagnosed with T2DM (43%) and 24 individuals without T2DM (57%). Of those with T2DM, 17 were female and one was male. Within the LFD group there were 11 individuals with T2DM (48%) and 12 individuals without T2DM (52%), and in the MedDiet group seven individuals had T2DM (37%) and 12 individuals did not have T2DM (63%).

4.8.3 Inflammatory Markers

There were no significant differences in inflammatory markers between participants diagnosed with and without T2DM in the LFD group at baseline or end-intervention, although inflammatory markers TNF-α, IL-6, leptin and resistin were all higher and adiponectin was lower (worse) in those with T2DM at both timepoints.

Participants with T2DM in the MedDiet group had significantly higher levels of circulating hs-CRP at baseline than participants without T2DM in the MedDiet group (4.2 ± 2.6mg/L vs 1.5 ± 1.1, p =0.035). hs-CRP decreased non-significantly in those with T2DM and increased in those without T2DM and the difference between those with and without diabetes was not significantly different post-intervention. Adiponectin was notably, albeit not significantly (p >0.05), lower at baseline in participants without diabetes who were randomised to the MedDiet arm and improved significantly following a MedDiet but not following the LFD diet (MD 10.5 ± 6.2µg/mL to 13.7 ± 8.6µg/mL, p =0.012, and LFD 18.4 ± 16.3µg/mL to 20.1 ± 25.2µg/mL, p =0.859). Aside from this, there were no significant differences in inflammatory markers or liver fat between individuals with and without T2DM in the MedDiet group.

There were no significant changes in inflammatory markers for individuals with T2DM in either diet group from baseline to end-intervention. Adiponectin increased noticeably, though non-significantly, in the MedDiet diet group (19.2 ± 11.4µg/mL to 23.6 ± 17.2 µg/mL, p =0.463) and increased to a lesser extent in the LFD group (16.1 ± 12.9 µg/mL to 18.7 ± 15.2µg/mL, p =0.314).

At baseline, participants without diabetes in the LFD group had significantly higher levels of hs-CRP than participants in the MedDiet group (4.0 ± 2.8mg/L vs 1.5 ± 1.1mg/L, p =0.025). There were no significant changes for hs-CRP in either diet group between baseline and end-intervention for participants without T2DM. At baseline, IL-6 was markedly though not significantly higher in the MedDiet group than the LFD group for participants without T2DM (5.3 ± 6.1pg/ml vs 18.3 ± 28.9pg/ml, p =0.608) and there were no significant changes in either diet group for IL-6.

There were no significant changes observed for TNF-α, leptin or resistin in either diet group for participants without diagnosed T2DM.

4.8.4 IHL and LSM

For participants in the LFD with T2DM, LSM was significantly higher than participants without T2DM at baseline (17.1 ± 19.3kPa vs 6.9 ± 4.5kPa, p =0.018) and end-intervention (16.7 ± 13.5kPa vs 6.5 ± 3.7kPa, p =0.027). There was no significant change in LSM in T2DM or non-T2DM participants in the LFD group from baseline to end-intervention. At baseline, LSM was significantly higher in those with T2DM compared to those without T2DM in the MedDiet group (10.4 ± 4.7kPa vs 6.3 ± 2.8kPa, p =0.027). At 12-weeks, LSM increased non-significantly in those with T2DM (10.4 ± 4.7 to 12.3 ± 6.8, p =0.753) and decreased non-significantly for those without T2DM (6.3 ± 2.8kPa to 5.3 ± 1.7kPa, p =0.091). LSM remained significantly higher in those with T2DM compared to those without T2DM at the end of the intervention (12.3 ± 6.8 vs 5.3 ± 1.7, p =0.05).

At baseline, participants with T2DM who were randomised to the LFD group had significantly lower IHL content than diabetic participants in the MedDiet group (6.0 ± 5.4% vs 16.2 ± 6.0%, p =0.022). Both groups experienced a similar reduction in IHL (%), though neither changes were significant (p >0.05). At the end-intervention timepoint the LFD diabetic group still had a significantly lower IHL content than the MedDiet diabetic group (3.9 ± 3.4% vs 13.6 ± 5.2, p =0.011).

4.8.5 Blood Biomarkers

In the LFD group, glucose was significantly higher in individuals with T2DM at baseline (7.9 ± 2.2mmol/L vs 5.6 ± 0.7mmol/L, p =0.003) and insulin and HOMA-IR were non-significantly higher in those with T2DM compared to those without T2DM. In the MedDiet group, glucose and HOMA-IR were markedly higher in individuals with T2DM than those without T2DM at baseline (6.7 ±

2.4 vs 5.3 ± 0.5, p =0.16), which became significantly higher at end intervention (6.5 ± 1.5 vs 5.3 ± 0.6, p =0.028).

Of those with diagnosed T2DM, insulin and HOMA-IR significantly decreased following a LFD (23.2 ± 12.8mIU/L to 19.5 ± 14.2mIU/L, p =0.028, and 8.6 ± 6.5 to 6.9 ± 6.01, p =0.008, respectively). The MedDiet group also experienced a decrease in insulin and HOMA-IR (21.1 ± 12.4mIU/L to 16.8 ± 12.4mIU/L, p =0.686, and 6.5 ± 4.6 to 4.8 ± 3.5, p =0.357, respectively), although the changes were not statistically significant, they are clinically meaningful. In participants without T2DM, fasting insulin decreased non-significantly in the LFD group (17.1 ± 11.8mIU/L to 14.1 ± 8.4mIU/L, p =0.075), and a slight non-significant increase in the MedDiet group (13.6 ± 4.9mIU/L to 14.8 ± 6.5mIU/L, p =0.646). For participants without T2DM, liver enzymes ALT and AST decreased significantly in the LFD group (71.9 ± 40.5U/L to 52.3 ± 19.3U/L, p =0.029, and 46.4 ± 23.6U/L to 35.5 ± 12.4U/L, p =0.033, respectively) and increased non-significantly in the MedDiet group (58.3 ± 28.8U/L to 72.3 ± 44.0U/L, p =0.343, and 30.2 ± 12.5U/L to 38.5 ± 26.7U/L, p =0.284, respectively).

4.8.6 Anthropometry and Body Composition

Individuals with T2DM in the LFD group had a significantly greater FM than individuals without T2DM at baseline (44.2 ± 5.9% vs 37.8 ± 8.4%, p =0.048), this difference did not remain at end-intervention (40.4 ± 8.4% vs 37.8 ± 7.6, p =0.473). For the MedDiet group, FM was significantly higher for those with T2DM than those without T2DM at baseline (45.5 ± 6.7% vs 35.4 ± 5.9%, p =0.003) and end intervention (45.7 ± 6.4% vs 35.5 ± 5.9%, p =0.004), but did not change from baseline to end-intervention within the diabetes groups.

At baseline, VF was similar for individuals with and without diabetes in the LFD group. VF significantly decreased in both T2DM (4.5 ± 2.3L to 1.2 ± 0.5L, p =0.018) and non-T2DM participants (4.4 ± 1.4L to 1.8 ± 0.6L, p =0.005) and by end intervention those with T2DM had significantly lower VF than those without T2DM (p =0.031).

4.8.7 Adherence to a Mediterranean Diet

There were no significant differences in PREDIMED scores between individuals with and without T2DM in the LFD and MedDiet groups. For individuals with diagnosed T2DM at baseline, PREDIMED scores for dietary adherence were significantly greater in the MedDiet arm compared to the LFD arm (7.3 ± 1.9 vs 4.5 ± 1.9, p =0.012, respectively). From baseline to end-intervention, Mediterranean Diet adherence increased significantly in the LFD group (4.5 ± 1.9 to 6.2 ± 1.3, p =0.014) and increased non-significantly in the MedDiet group (7.3 ± 1.9 to 8.8 ± 2.6, p =0.233),

though the scores were still significantly greater in the MedDiet group at end-intervention ($p = 0.023$).

For individuals without T2DM, PREDIMED scores significantly improved from baseline to end-intervention following a LFD (5.4 ± 1.6 to 6.5 ± 1.9, $p = 0.035$) and a MedDiet (6.0 ± 1.9 to 9.3 ± 1.5, $p < 0.001$). Change in PREDIMED scores was significantly greater in the participants without diabetes in the MedDiet compared with the LFD (3.3 ± 1.7 vs 1.1 ± 1.6, $p = 0.003$).

Table 4.22. Mean values for inflammatory markers, liver outcomes and key biochemical, anthropometric and body composition variables at baseline and end-intervention timepoints, by dietary intervention arm (LFD vs. MedDiet) and diagnosis of T2DM

	T2DM (n=18)‡					Non-T2DM (n=24)					P[c]	
	Control LFD (n=11)		Intervention MedDiet (n=7)			Control LFD (n=12)		Intervention MedDiet (n=12)				
	mean	SD	mean	SD	P[a]	mean	SD	mean	SD	P[b]	LFD	MedDiet
Inflammatory markers												
hs-CRP(mg/L)												
Baseline	3.69	2.67	4.16	2.64	0.681	3.95	2.82	1.45	1.08	0.025*	0.832	0.035*
End Intervention	3.71	2.42	3.32	2.56	0.876	3.33	2.85	1.57	1.08	0.169	0.766	0.093
TNF-α (pg/ml)												
Baseline	7.75	8.79	3.81	2.33	0.246	4.35	0.99	3.81	1.97	0.316	0.230	0.995
End Intervention	5.25	2.45	3.92	1.32	0.388	3.97	1.56	4.19	2.19	0.755	0.160	0.788
IL-6 (pg/ml)												
Baseline	10.48	11.81	9.23	12.94	0.791	5.29	6.11	18.32	28.95	0.608	0.210	0.446
End Intervention	12.67	10.51	10.00	14.18	0.689	10.43	17.02	19.87	29.81	0.410	0.732	0.458
Adiponectin (µg/mL)												
Baseline	16.10	12.86	19.16	11.35	0.596	18.40	16.30	10.49	6.21	0.379	0.717	0.098
End Intervention	18.73	15.17	23.60	17.18	0.607	20.12	25.23	13.71••	8.59	0.671	0.885	0.230
Leptin (ng/ml)												
Baseline	21.58	15.42	16.35	10.39	0.596	15.73	11.48	11.84	9.59	0.26	0.324	0.350
End Intervention	18.23	14.02	17.41	12.38	0.955	15.55	12.94	11.76	8.85	0.378	0.655	0.279
Resistin (ng/mL)												
Baseline	48.44	28.03	35.23	14.06	0.479	34.77	13.70	38.75	13.00	1.000	0.162	0.588
End Intervention	47.57	25.39	42.49	21.12	0.607	32.57	14.83	38.67	12.43	0.266	0.105	0.632
Liver Outcomes												
Liver Fat (%)												
Baseline	6.01	5.40	16.23	6.03	0.022*	13.29	15.94	13.48	9.14	0.984	0.608	0.935

End Intervention	3.85	3.38	13.60	5.17	0.011*	15.72	19.78	12.79	9.15	0.778	0.335	0.819
LSM (kPa)												
Baseline	17.09	19.27	10.36	4.72	0.724	6.99	4.46	6.25	2.76	0.887	0.018*	0.027*
End Intervention	16.71	13.45	12.32	6.77	0.852	6.50	3.68	5.29	1.69	0.651	0.027*	0.050*
Biochemistry												
Glucose (mmol/L)												
Baseline	7.94	2.23	6.74	2.39	0.085	5.66	0.77	5.28	0.54	0.143	0.003*	0.160
End Intervention	7.66	2.12	6.52	1.52	0.145	6.08	2.43	5.30	0.66	0.319	0.136	0.028*
Insulin (mIU/L)												
Baseline	23.15	12.76	21.13	12.38	0.596	17.13	11.82	13.63	4.97	0.713	0.253	0.168
End Intervention	19.53•	14.24	16.80	12.39	0.776	14.07	8.36	14.81	6.45	0.478	0.283	0.655
HOMA-IR												
Baseline	8.64	6.53	6.49	4.56	0.479	4.53	3.78	3.20	1.21	0.410	0.076	0.107
End Intervention	6.87•	6.01	4.75	3.45	0.607	4.44	5.16	3.47	1.54	0.932	0.331	0.028*
ALT (U/L)												
Baseline	50.09	30.62	47.00	17.22	0.930	71.92	40.48	58.25	28.81	0.590	0.162	0.363
End Intervention	39.78	21.73	49.50	24.90	0.607	52.25••	19.34	72.33	44.01	0.478	0.181	0.259
AST (U/L)												
Baseline	36.82	19.05	34.57	13.14	1.000	46.42	23.59	30.17	12.49	0.052	0.298	0.477
End Intervention	32.22	19.28	42.00	31.23	0.776	35.50••	12.44	38.50	26.70	0.514	0.641	0.807
GGT (U/L)												
Baseline	90.36	73.28	119.86	110.31	0.860	159.92	160.55	72.67	39.79	0.114	0.203	0.312
End Intervention	67.33	46.99	137.33	105.39	0.224	116.08	83.39	88.92	83.43	0.319	0.133	0.302
ALP (U/L)												
Baseline	87.45	18.53	104.00	40.11	0.425	98.58	42.97	84.17	14.89	0.479	0.437	0.249
End Intervention	92.00	22.92	113.33	41.99	0.388	95.83	27.11	88.58	22.08	0.551	0.736	0.222
Weight (kg)												
Baseline	87.36	32.49	90.30	25.01	0.842	92.03	14.95	86.22	19.53	0.422	0.670	0.697
End Intervention	80.41	23.89	91.83	25.99	0.397	89.82	11.92	88.00	22.14	0.806	0.249	0.748
BMI (kg/m^2)												

Baseline	32.90	8.78	34.10	7.20	0.766	32.57	5.14	30.16	3.75	0.204	0.913	0.132
End Intervention	30.33	5.98	33.99	7.64	0.318	32.01	4.07	30.78	4.13	0.471	0.454	0.258
WC (cm)												
Baseline	110.15	25.26	109.83	18.55	0.724	107.45	11.40	102.35	12.00	0.291	0.751	0.298
End Intervention	101.81	17.75	110.28	18.51	0.456	105.51	7.51	104.23	13.77	0.266	0.570	0.443
Fat Mass (%)												
Baseline	44.20	5.93	45.51	6.72	0.669	37.78	8.41	35.41	5.98	0.436	0.048*	0.003*
End Intervention	40.38	8.42	45.67	6.44	0.216	37.80	7.66	35.46	5.86	0.410	0.473	0.004*
Visceral Fat (L)												
Baseline	4.46	2.85	3.18	1.36	0.524	4.42	1.39	3.19	1.65	0.030*	0.967	0.988
End Intervention	1.21•	0.55	2.12	2.50	0.689	1.78••	0.57	1.61••	0.50	0.410	0.031*	0.640
Adherence to a Mediterranean Diet												
PREDIMED Scores at Baseline	4.50	1.96	7.29	1.98	0.012*	5.42	1.62	6.00	1.91	0.428	0.243	0.180
PREDIMED Scores at End Intervention	6.22•	1.30	8.83	2.64	0.023*	6.50••	1.88	9.33	1.50••	<0.001*	0.694	0.611
Change in PREDIMED Score	1.78	1.72	1.67	3.01	0.928	1.08	1.56	3.33	1.72	0.003*	0.346	0.151

T2DM, Type 2 Diabetes Mellitus; LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; BMI, Body Mass Index; WC, waist circumference; HOMA-IR, homeostatic model assessment-insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LSM, liver stiffness measure; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6. All data presented as mean ± SD. * indicates significance ($p < 0.05$). ǂ indicates n=18 at baseline and n=15 at end-intervention (2 dropouts in the LFD group and 1 dropout in the MedDiet). P^a p-values for comparing differences between participants diagnosed with T2DM in the LFD and MedDiet groups at baseline and at end intervention; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences between participants without diagnosed T2DM in the LFD and MedDiet groups at baseline and at end intervention; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. • p-values (≤0.05) for comparing differences within each diet group for participants diagnosed with T2DM from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. •• p-values (≤0.01) for comparing differences within each diet group for participants without diagnosed T2DM from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values comparing differences between participants with and without diabetes within each diet group at baseline and end intervention; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data.

4.8.8 Analysis of Diabetes Status in the Pooled Patient Cohort, Irrespective of Diet Group Allocation

Section One of this chapter identified that diet quality improved in participants assigned to both the MedDiet and LFD intervention, and while there were improvements in various outcomes within each group, the overall effect of a "healthy" diet may have been more dependent on the individual's severity of disease (or disease status). Additionally, the number of participants with more severe disease status (i.e., diagnosed with both NAFLD and T2DM) was disproportionate between diet groups. In order to further explore the effect of severity of disease status on response to dietary intervention, data was pooled and analysed in participants diagnosed with T2DM. This analysis was conducted to compare features of participants diagnosed with both diabetes and NAFLD to those diagnosed with NAFLD only (without diabetes), and to assess differences in inflammatory markers, liver outcomes and key biochemical and anthropometric measures of both groups of participants (T2DM vs non-T2DM) while following either "healthy" dietary intervention. Mean values for these outcomes at pre- and post-intervention timepoints are presented in **Table 4.23**.

4.8.8.1 Differences between individuals with and without diagnosed T2DM in the pooled MEDINA Cohort at Baseline

Of the inflammatory markers at baseline; hs-CRP, TNF-α, leptin and resistin were all greater in the T2DM group than the non-T2DM group, although no significant differences were observed between groups. Adiponectin was also greater in the T2DM group than the non-T2DM group at baseline, albeit not significantly. Interestingly, measures including weight and liver enzymes ALT, AST, GGT, and liver fat were all non-significantly lower in the group with diagnosed T2DM than those without T2DM at baseline. These findings may be attributable to medication in this subset.

Prior to any dietary intervention being administered, individuals diagnosed with T2DM had significantly greater levels of FM (44.7 ± 6.1% vs 36.6 ± 7.2%, p =0.0005), glucose (7.5 ± 2.3mmol/L vs 5.5 ± 0.7mmol/L, p =0.001) and HOMA-IR (7.8 ± 5.8 vs 3.9 ± 2.8, p =0.003) than those without T2DM. Similarly, individuals with T2DM had a greater BMI (33.4 ± 8.0kg/m^2 vs 31.4 ± 4.6kg/m^2, p =0.351), WC (110.0 ± 22.3cm vs 104.9 ± 11.7cm, p =0.990), VF (4.0 ± 2.4L vs 3.8 ± 1.6, p =0.933) and fasting insulin (22.4 ± 12.3mIU/L vs 15.4 ± 9.1mIU/L, p =0.052) than those without T2DM, although these differences were not significant. These findings were expected in participants with diabetes and representative of the population established in previous literature. Elevated HOMA-IR, BMI and VF are well-known risk factors for diabetes.

4.8.8.2 *Effects of improved diet quality following diet intervention on Anthropometry, Haemodynamic and Body Composition measures in individuals with and without diagnosed T2DM (in the pooled cohort)*

A number of changes were observed for participants with diagnosed T2DM and without T2DM from baseline to end-intervention, with an improved diet quality post-intervention.

Insulin and HOMA-IR decreased significantly in those with T2DM (22.4 ± 12.3mIU/L to 18.4 ± 13.1mIU/L, p =0.022, and 7.8 to 5.8, p =0.003, respectively), while a non-significant change was observed for fasting insulin for those without T2DM (15.4mIU/L to 14.4mIU/L) and no change was seen for HOMA-IR in those without T2DM (3.9 to 4.0, p =0.903). WC decreased significantly in the T2DM group (110.0 ± 22.3cm to 105.2 ± 17.9cm, p =0.047) and remained unchanged in the non-T2DM group (104.9 ± 11.7cm to 104.9 ± 10.8cm, p =0.71). VF decreased significantly in both the T2DM and non-T2DM groups (3.9 ± 2.4L to 1.6 ± 1.6L, p =0.028, and 3.8 ± 1.6L to 1.7 ± 0.5L, p =0.0005, respectively). Both groups significantly improved their adherence to a MedDiet based on PREDIMED scores (T2DM 5.7 ± 2.4 to 7.8 ± 2.3, p =0.009, vs Non-T2DM 5.7 ± 1.8 to 7.9 ± 2.2, p =0.0005).

4.8.8.3 *Differences between individuals with and without diagnosed T2DM in the pooled MEDINA Cohort at End-Intervention*

LSM was significantly greater in the T2DM group than the non-T2DM group at the end-intervention timepoint (14.8 ± 10.9kPa vs 5.8 ± 2.8kPa, p =0.001), although was relatively greater to begin with at baseline (14.47 ± 15.42kPa vs 6.62 ± 3.65kPa, p =0.062). LSM did not change from pre- to post-intervention in the diabetic group (14.47 ± 15.42kPa to 14.83 ± 10.96kPa, p =0.221), and dropped by less than one unit in the non-diabetics (6.62 ± 3.65kPa to 5.87 ± 2.82kPa, p =0.299).

At end-intervention in the pooled cohort, individuals with T2DM had significantly higher FM (42.5 ±7.9% vs 36.6 ± 6.8%, p =0.0005) and levels of glucose (7.2 ± 1.9mmol/L vs 5.7 ± 1.8mmol/L, p =0.001) than individuals without T2DM – which did not change significantly in either group over the intervention period. Interestingly, VF decreased to a greater extent in diabetics compared to non-diabetics and this difference was significantly different at the end intervention timepoint (1.8 ± 1.6L vs 1.7 ± 0.5L, p =0.005). 3

Table 4.23. Mean values for inflammatory markers, liver outcomes and key biochemical, anthropometric and body composition variables at baseline and end-intervention timepoints, categorised diagnosis of T2DM

	T2DM (n=18)ǂ		Non-T2DM (n=24)			P[b]	
	mean	SD	mean	SD	P[a]	T2DM	Non-T2DM
Inflammatory markers							
hs-CRP(mg/L)							
Baseline	3.89	2.58	2.81	2.51	0.171	0.838	0.376
End Intervention	3.55	2.37	2.57	2.39	0.163		
TNF-α (pg/ml)							
Baseline	6.22	7.16	4.06	1.57	0.563	0.245	0.745
End Intervention	4.72	2.12	4.08	1.87	0.484		
IL-6 (pg/ml)							
Baseline	10.00	11.89	12.09	21.92	0.299	0.394	0.108
End Intervention	11.60	11.69	15.15	24.23	0.466		
Adiponectin (µg/mL)							
Baseline	17.29	12.04	14.27	12.50	0.372	0.281	0.201
End Intervention	20.68	15.59	16.92	18.72	0.399		
Leptin (ng/ml)							
Baseline	19.55	13.60	13.70	10.48	0.134	0.46	0.543
End Intervention	17.91	12.93	13.66	11.01	0.296		
Resistin (ng/mL)							
Baseline	43.30	24.00	36.84	13.19	0.537	0.57	0.715
End Intervention	45.54	23.12	35.62	13.74	0.283		
Liver Outcomes							
Liver Fat (%)							
Baseline	11.12	7.62	13.37	12.59	0.650	0.198	0.555
End Intervention	8.18	6.49	14.42	15.15	0.281		
LSM (kPa)							
Baseline	14.47	15.42	6.62	3.65	0.062	0.221	0.299
End Intervention	14.83	10.96	5.87	2.82	0.001*		

	T2DM (n=18)ǂ		Non-T2DM (n=24)			P[b]	
	mean	SD	mean	SD	P[a]	T2DM	Non-T2DM
Biochemistry							
Glucose (mmol/L)							
Baseline	7.47	2.30	5.47	0.68	0.001*	0.124	0.984
End Intervention	7.20	1.93	5.69	1.78	0.001*		
Insulin (mIU/L)							
Baseline	22.37	12.28	15.38	9.05	0.052	0.022*	0.366
End Intervention	18.44	13.14	14.44	7.32	0.765		
HOMA-IR							
Baseline	7.80	5.79	3.86	2.83	0.003*	0.003*	0.903
End Intervention	6.03	5.10	3.95	3.75	0.062		
ALT (U/L)							
Baseline	48.89	25.66	65.08	35.06	0.112	0.198	0.433
End Intervention	43.67	22.71	62.29	34.79	0.091		
AST (U/L)							
Baseline	35.94	16.60	38.29	20.24	0.839	0.315	0.486
End Intervention	36.13	24.19	37.00	20.43	0.502		
GGT (U/L)							
Baseline	101.83	87.59	116.29	122.76	0.431	0.173	0.444
End Intervention	95.33	80.55	102.50	82.75	0.539		
ALP (U/L)							
Baseline	93.89	28.96	91.38	32.30	0.656	0.615	0.550
End Intervention	100.53	32.36	92.21	24.46	0.743		
Anthropometry and Body Composition							
Weight (kg)							
Baseline	88.51	29.05	89.13	17.27	0.937	0.226	0.927
End Intervention	84.97	24.51	88.91	17.41	0.561		
BMI (kg/m^2)							

	T2DM (n=18)ǂ		Non-T2DM (n=24)			P^b	
	mean	**SD**	**mean**	**SD**	**P^a**	**T2DM**	**Non-T2DM**
Baseline	33.36	8.00	31.36	4.57	0.351	0.201	0.967
End Intervention	31.79	6.69	31.39	4.06	0.836		
WC (cm)							
Baseline	110.02	22.29	104.90	11.74	0.990	0.047*	0.71
End Intervention	105.20	17.91	104.87	10.87	0.558		
Fat Mass (%)							
Baseline	44.71	6.09	36.59	7.24	<0.001*	0.404	0.977
End Intervention	42.49	7.91	36.63	6.78	<0.001*		
Visceral Fat (L)							
Baseline	3.97	2.40	3.75	1.62	0.933	0.028*	<0.001*
End Intervention	1.57	1.62	1.69	0.53	0.005*		
Adherence to a Mediterranean Diet							
PREDIMED Scores at Baseline	5.65	2.37	5.71	1.76	0.925	0.009*	<0.001*
PREDIMED Scores at End Intervention	7.27	2.28	7.92	2.21	0.383		
Change in PREDIMED Score	1.73	2.22	2.21	1.98	0.491		

T2DM, Type 2 Diabetes Mellitus; SD, Standard Deviation; BMI, Body Mass Index; WC, waist circumference; HOMA-IR, homeostatic model assessment-insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LSM, liver stiffness measure; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6. All data presented as mean ± SD. * indicates significance ($p < 0.05$). ǂ indicates n=18 at baseline and n=15 at end-intervention.

P^a p-values for comparing differences between participants diagnosed with or without T2DM at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing within-group differences for participants diagnosed with or without T2DM from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data.

4.9 Discussion

The primary aim of this chapter was to determine the effect of a MedDiet versus LFD on inflammatory markers in patients with NAFLD. The results of the study found that in patients with NAFLD, the MedDiet resulted in significant improvements in circulating serum adiponectin. There was no significant effect of the MedDiet or LFD on hs-CRP, TNF-α, IL-6, leptin and resistin. Secondary aims of this chapter were to assess liver outcome measures, biochemical markers and anthropometry, body and diet composition following the 12-week intervention. The results demonstrated that improvements in adiponectin levels observed following the MedDiet were accompanied by a significant reduction in VF in the absence of any significant weight loss.

There were no significant changes in inflammatory markers following the LFD, however participants in the LFD group showed greater improvements in HOMA-IR, liver enzymes (AST, ALT and GGT) and VF. Although not statistically significant, a noteworthy reduction of -4.0 ± 2.3 kg body weight occurred following the LFD, despite the advice given to maintain weight and no change advised to overall energy intakes. Diet quality and adherence to a Mediterranean-style dietary pattern significantly improved for all participants, regardless of diet group assignment. The whole NAFLD cohort reduced their intake of SFAs and refined grains, and increased intakes of MUFAs and whole grains. Participants in the MedDiet group consumed significantly more MUFAs and n-3 fatty acid eicosapentaenoic acid (EPA) during the intervention, likely in the form of EVOO and fish/seafood as per the diet recommendations. They also significantly reduced dietary intake of SFAs and added sugars. The LFD arm significantly reduced their consumption of total fat and added sugars. In the pooled study cohort, participants with a higher MedDiet adherence score had significantly lower levels of circulating hs-CRP, leptin and HOMA-IR.

The Mediterranean Diet and Inflammation

Chronic low-grade inflammation occurs as a result of the accumulation of excess adipose tissue and is considered an underlying pathophysiological feature in the development of NAFLD.[530] Of the inflammatory markers studied, hs-CRP, TNF-α, IL-6, leptin and resistin did not change over the 3-month MedDiet intervention period. However, adiponectin significantly improved following the MedDiet intervention. Adiponectin is an adipokine marker predominantly secreted from adipose tissue, known to be markedly reduced in visceral obesity and states of IR such as T2DM and NASH.[531] Low levels of circulating adiponectin, or hypoadiponectinemia, are negatively correlated with body fat, hepatic steatosis and fibrosis.[532] Consistent with the results of the present study, Sofi et al. (2010) also found that levels of adiponectin significantly increased in patients with NAFLD following long-term consumption of olive oil enriched with n-3 PUFA.[380] Additionally, Markova et al. (2016) saw a significant increase in adiponectin in their NAFLD cohort following a plant-protein based isocaloric diet, compared to an animal-protein isocaloric diet.[533] The results of the

present study highlight the change in circulating adiponectin in the absence of weight loss, though participants experienced a notable change in VF. This finding supports the notion that visceral adipose tissue regulates the secretion of adiponectin into the bloodstream, and not subcutaneous adipose tissue. Previous studies have reported the association between higher adiponectin levels and adherence to a Mediterranean-type diet, beyond variations in energy intake, exercise and body weight in obese and diabetic women.[505, 534] Mantzoros et al. (2006) suggested that these findings were not fully explained by confounders, and concluded that the association of adiponectin with the MedDiet was attributed to intakes of red wine, nuts and wholegrains.[505] In agreement with results of the present study, a large cross-sectional study reported the inverse relationship between adiponectin concentrations and central body fat, independent of sex and overall adiposity.[535] The anti-inflammatory and anti-oxidant properties of a MedDiet may mediate glucose and lipid metabolism and alter adipose tissue, consequently improving adipokine production, without causing weight change.[535] In a physically inactive and obese population, this effect of reducing VF and associated levels of adiponectin without inducing weight loss could be a sustainable and effective treatment.

Changes in the quality of dietary fats consumed in the MedDiet arm were significant and aligned with dietary recommendations provided to participants; specifically, a significant reduction in SFAs and significant increase in MUFAs were observed. Consistently, serves of 'oil equivalents' (likely to be predominantly EVOO) increased significantly in the MedDiet group. Polyunsaturated FAs – particularly very long chain n-3 fatty acids − also increased following a MedDiet, albeit not significantly. The type and amount of dietary fat consumed through diet is important in modulating adipose tissue function, attenuating inflammation and regulating metabolism.[536] Each of the different types of fatty acids are structurally unalike with functional differences. The role of SFAs in promoting a pro-inflammatory and insulin-resistant state in metabolic disorders is well known and associated with increased rates of obesity, NAFLD and CVDs.[124] Indeed, there is strong evidence behind the molecular processes and detrimental effects of SFAs.[333] In contrast, MUFAs and PUFAs are identified as having anti-inflammatory, insulin-sensitizing properties and are used in the context of obesity-related inflammation and disease research as a potential therapeutic target.[512, 525, 537] Two forms of long chain n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), mostly found in fish and fish oil lead to improvements in adipose tissue function thereby reducing inflammation.[536] MUFAs are more readily found in olive oil, fruits such as avocado and nuts, and reduce pro-inflammatory cytokine secretion from adipocytes.[536] What remains unclear, is if the replacement of SFA with another fatty acid (MUFA or n-3 PUFA) will resolve chronic low-grade inflammation and an insulin-resistant environment. Spadaro et al. (2008) conducted a 6-month randomized trial in patients with NAFLD, who were randomised to follow the American Heart Association (AHA) Healthy Diet Guidelines or to follow the same AHA diet with the addition of 1g PUFAs supplement (capsule) twice per day. Researchers found that the PUFA treatment reduced IHL (%) content, ALT, IR and decreased circulating serum levels of TNF-

α.[538] Another study supplemented n-3 PUFA enriched EVOO to a small group of patients with NAFLD for one year, after which they showed significantly reduced liver enzymes, triglycerides and improved levels of circulating adiponectin compared to controls.[380] The mechanisms by which MUFAs (in EVOO) and PUFAs (especially n-3 FAs) exert beneficial their effect is mainly attributed to the up-regulation of hepatic lipolysis and fat oxidation, and down-regulation of hepatic lipogenesis.[285] Although these dietary elements are considered to have anti-inflammatory and anti-fibrotic properties, there were no significant reductions in inflammatory cytokines following the MedDiet in the present study. A possible explanation for this might be that while dietary changes were found to be significant, they may not have been substantial enough to promote an anti-inflammatory change in biomarkers of inflammation or the time of intervention was not adequate for noticeable inflammatory changes to occur in this population. Another possible explanation is the limited sample size of this study lacked statistical power to see a change in impervious inflammatory markers. A larger cohort of 150-300 participants with NAFLD are needed in order to re-assess the effect of the MedDiet on inflammatory markers in a powered sample. Future analyses should follow participants for a longer period of time to show efficacy and to determine sustainability of prescribed dietary interventions.

In the MedDiet arm of this study, MUFA consumption increased through increased consumption of MUFA-rich foods (such as EVOO) as prescribed to participants. EVOO is a key component of the MedDiet pattern,[281, 285, 306] and was recommended in doses of minimum 3–4 tablespoons (60–80 mL) per day to participants randomised to the MedDiet group of this study.[345, 529] A recent systematic review and meta-analysis investigated the cardioprotective effect of high versus low polyphenol olive oil in clinical trials, found that the consumption of high polyphenol olive oil generally reported improvements in inflammation, oxidative stress and endothelial function.[539] The benefits of high polyphenol olive oil were independent of high MUFA content. Virgin and extra virgin olive oil (considered higher grade olive oil) have a higher polyphenol and phytochemical content which is altered when the olive oil is refined or heated.[540] The anti-inflammatory mechanism of both MUFAs and PUFAs is thought to begin with the activation of PPARs, which stimulate oxidation of FFAs and decreases inflammation, IR and gene expression involved in hepatic DNL, therefore reducing hepatic steatosis.[541] A study of 28 patients with coronary heart disease found that supplementation of 50mL virgin olive oil daily over two periods of 3-weeks resulted in a significant decrease in plasma IL-6 and CRP compared to supplementation with refined olive oil.[510] In healthy adults asked to consume 50mL daily of either EVOO, corn oil, soy oil or cod liver oil, plasma concentrations of TNF-α reduced significantly with EVOO, soy and cod liver oil consumption.[542] One study by Nigam et al. (2014) focussed on dietary intervention with olive or canola oil in comparison with commonly used refined oil soyabean/safflower oil (control).[543] It found that olive and canola oil led to a significant reduction in IR, lipids and grading of fatty liver compared with the control, however olive oil had a significantly greater reduction for IR than canola oil.[543] Furthermore, Ryan et al. (2013) implemented a randomized-cross over trial investigating the effect

of a MedDiet compared to a low-fat/high-carbohydrate diet (control group) and found that the MedDiet resulted in significantly improved IR and hepatic steatosis compared to the control group, and this finding was independent of weight loss.[499] Participants received precooked meals and additional food items to improve compliance to diet. Results from the trial indicated that fat restriction may not be the key dietary element in treating NAFLD and in particular, additional investigation into the type of dietary fat is required. Considering that weight loss is difficult to maintain in this population and results indicated that weight loss did not in fact influence the reduction in hepatic steatosis and insulin, studies should be investigating non-weight loss centred diets.[499] The anti-inflammatory and insulin-lowering effects of EVOO, independent of weight loss, have been observed in outlined studies. The MedDiet is a moderate to high fat diet with EVOO the main dietary fat, however further research and dietary intervention is required to identify the mechanism of action for alleviation of NAFLD pathologies. Some investigators recommend moderate MUFA consumption of 20 g/day, though there is still some controversy between the results of diet intervention studies.[540] Hence, additional studies are required to fully understand effects of MUFA intake and to establish a recommended amount.

Results of this study demonstrated that diet quality, composition and overall adherence to a MedDiet (as determined by PREDIMED scores) improved for both the MedDiet and LFD arms over the 3-month intervention period. Both groups significantly decreased the amount of added sugars consumed per day. Sugar-sweetened foods and beverages are known to play a role in the development of NAFLD by raising triglycerides, blood glucose and promoting IR. This can stimulate hepatic DNL and lipid peroxidation, and increase fatty deposits in the liver and hepatic inflammation.[544, 545] Studies show that individuals who consume more added sugar in their diet tend to lead sedentary lifestyles, consume less fibre and greater amounts of saturated and trans fat, and have an overall high-caloric diet, including more fructose and simple carbohydrates.[544] Zelber-Sagi et al. (2007) found that NAFLD patients were consuming higher intakes of soft drinks and meat, and a lower intake of fish rich in omega-3s.[546] Soft drinks and other sweetened beverages, as well as processed foods are made up primarily of fructose. Fructose intake contributes to the inflammatory process, at the point of liver fat accumulation where hepatocytes are vulnerable to cellular stress, activation of the NFκB pathways produce pro-inflammatory cytokines such as TNF-α by Kupffer cells and further increases oxidative stress.[547]A small number of studies have investigated the effect of dietary sugar restriction in NAFLD; one study in twelve obese adolescents diagnosed with NAFLD reported modest reductions in fructose and high-GI carbohydrate intake significantly reduced body fat, IR and liver enzymes.[548] A randomised-controlled trial by Campos et al. (2015) provided further evidence that replacement of sugar-sweetened beverages (high fructose) with non-caloric artificially-sweetened beverages can reduce IHL content, most significantly in those with high IHL content.[545] Moreover, following a 4-week randomised double-blind study of calorie-matched fructose versus glucose beverages, twenty-four overweight Hispanic-American adolescents with hepatic steatosis did not have altered hepatic fat or body

weight in either group. However, the glucose beverage group significantly improved LDL oxidation, insulin sensitivity and hs-CRP.[549] Another study in a larger cohort of 427 patients with NAFLD, reported that fructose consumption was associated with reduced hepatic steatosis and increased hepatic fibrosis, lobular inflammation and ballooned hepatocytes.[550] Current guidelines for the dietary management of NAFLD promote energy restriction and exclusion of dietary components such as processed foods and food or beverage products high in added fructose that are known to be NAFLD-promoting.[494]

Compliance to a MedDiet was also observed through a significant reduction in carbohydrate intake in the MedDiet arm and overall cohort; particularly, a reduction in the amount of refined grains consumed and an increase in wholegrain consumption. Epidemiological studies have consistently associated high wholegrain consumption with decreased risk of obesity, diabetes and CVDs, compared to little or no wholegrain comsumption.[551] In NAFLD, similar observations have been made for wholegrain intake when compared with refined grain intake. Reductions in body fat, fasting C-peptide and insulin have been observed as result of replacing refined grains with wholegrains, likely due to the reduction in energy intake (lower energy density) and amount of carbohydrate consumed.[551] Some studies have reported a correlation between the intake of wholegrains and improvement in circulating inflammatory markers, though this finding is not wholly confirmed by the literature The anti-inflammatory effects of wholegrains stem from high amounts of bioactive properties including fibre, vitamin B, vitamin E, antioxidants and phytoestrogens.[552] A recent meta-analysis found conflicting results of wholegrain consumption on inflammatory markers CRP, IL-6 and TNF-α.[553] Pooled effect sizes showed no significant effect of wholegrain consumption on circulating serum CRP, unless an individual had elevated serum CRP or was following an isocaloric diet. There was no significant effect of wholegrain consumption on serum IL-6 concentrations in healthy populations, however a significant effect was found for unhealthy individuals. There was no significant effect in studies for TNF-α.[553] It should be noted that the outcomes of such studies may be highly dependent on the study population and design. While the mechanisms of wholegrains require further investigation, in conjunction with a 'healthy' diet can lead to metabolic (and inflammatory) improvements. Wholegrains are an essential component of the MedDiet and their ability to interact synergistically with other components of the diet to produce anti-inflammatory effects may be an effective strategy for management of NAFLD.

Health benefits of a MedDiet pattern are often analysed through adherence to each of the functional components of the diet. In the present study, participants who were complying with more components of a MedDiet and therefore had a higher MedDiet adherence score had significantly lower levels of circulating hs-CRP, leptin and HOMA-IR. In a Greek province called Attica, researchers found that healthy adults who adhered to a more traditional MedDiet had lower levels of circulating plasma CRP, IL-6 and homocysteine.[518] Another study conducted in healthy women reported an inverse correlation between a prudent dietary pattern, similar to a MedDiet pattern, and

CRP.[554] The same study found that a Western diet, high in red meat, commercial sweets, fries and refined grain intake, was positively correlated with CRP and IL-6.[554] A study in the Mediterranean region of the Balearic Islands reported interesting results in healthy adolescents compared to adults adhering to a Mediterranean diet; adult male participants with higher adherence to a MedDiet pattern had higher levels of adiponectin and lower leptin, TNF-α and hs-CRP compared with adult males with lower adherence.[555] These results were not observed in younger males.[555] Interestingly, in adult females the only inflammatory marker associated with higher adherence to a MedDiet pattern was hs-CRP and for female adolescents both hs-CRP and leptin.[555] There are only a few studies assessing the effects of dietary intervention and especially Mediterranean diet and weight loss on circulating leptin.[556, 557] Studies supporting the notion that good adherence to the MD produces anti-inflammatory and antioxidant effects and overall cardiometabolic benefits are growing. Since many of the evidenced studies were conducted in Mediterranean populations, there is a need for substantial data to be collected in non-Mediterranean populations. Larger cross-sectional or cohort studies are required.

Weight loss and the Low-Fat Diet

Following the LFD intervention there was a significant reduction in levels of fasting insulin and HOMA-IR. A significant reduction in liver enzymes ALT, AST and GGT was also observed, and a non-significant albeit clinically relevant decrease in body weight of -4.0 ± 2.3kg (3.5%) was observed in the LFD arm only. Weight loss was not an intentional outcome of this trial, although it can be partially explained by the ~1,000kJ energy deficit observed in this study group over the 12-week intervention. Current data for the management of NAFLD suggest that weight loss (aimed at approx. 7-10% body weight) is the driving factor for reduction in hepatic steatosis, and is therefore recommended as the mainstay therapy in NAFLD.[494] In fact, the magnitude of change in hepatic steatosis and liver enzymes has been strongly correlated to the degree of weight reduction experienced.[558] A systematic review by Thoma et al. (2012), revealed that a range of lifestyle modifications often involving energy restriction, with and without increased physical activity, and weight reduction were most effective in reducing IHL (%), circulating liver enzymes, and improving measures of glucose control and/or insulin sensitivity in patients with NAFLD.[559] They further defined weight reductions of 4–14% resulted in statistically significant relative reductions of IHL 35–81%.[559] A calorie-restricted diet or LFD were frequently used to achieve weight loss in NAFLD; De Luis et al. (2010) examined the effect of a hypocaloric LFD and a hypocaloric low-CHO diet and found that both diets were effective in improving IR, though the LFD improved IR to a greater extent.[560] Again, this change was considered to be driven by weight loss as both groups lost an average of 3.5% in weight.[560] Authors did not evaluate IHL content, however a decrease in total and LDL-cholesterol was associated with the restriction of dietary fat intake and its impact on NAFLD physiology.[560] Weight loss remains the primary treatment for patients with NAFLD at

present, the sustainability of which in a metabolically disordered population is still a concern for many treating practitioners.

In the present study, the reduction of liver enzymes ALT, AST and GGT and insulin resistance (HOMA-IR) experienced by participants in the LFD group appeared to be sensitive to the restriction of dietary fats. In the LFD group, total fat intake (as percent total energy) decreased significantly and saturated fat intake (as percent total energy) decreased non-significantly. Indeed, literature surrounding the NAFLD population has identified high SFA intake as a characteristic of disease pathogenesis and progression.[246] Dietary intake of foods high in SFA have consistently been reported to be high in individuals with NAFLD.[246] Elevated liver enzymes and insulin resistance are two main risk factors for NAFLD and are evidently influenced by SFA intake. Researchers have previously hypothesised that PUFA are more rapidly oxidised than SFA.[561] Increased oxidation of fatty acids from PUFA ingestion would result in less fatty acids available for esterification to TG and potential accumulation of liver fat.[562] A systematic review and meta-analysis of eight randomised controlled trials found that replacing SFA with PUFA reduced the occurrence of cardiovascular events by 19%.[537] Each 5%E increase in PUFA consumption reduced cardiovascular risk by 10%.[537] Similarly, prospective cohort and observational studies have associated increased risk of NAFLD and cardiovascular events with SFA consumption.[540] Many researchers have claimed the importance of not only SFA reduction, but the replacement of SFA consumption with PUFA.[562] Large-scale dietary intervention trials are required to confirm the beneficial effects of SFA replacement with PUFA on insulin resistance, liver outcomes and cardiovascular risk factors in NAFLD.

Improvement in Diet Quality (pooled cohort) and Impact of Diabetes

One of the main findings of this study stemmed from the subgroup analysis of participants with NAFLD who were diagnosed with or without diabetes. The analysis was conducted based on the presence of individuals with T2DM across diet groups and the potential for these participants to potentially respond to dietary intervention based on a more severe metabolic and liver disease phenotype. The pooled cohort analysis between participants with and without T2DM showed that regardless of diet group, intervening with a "healthy" dietary intervention that improves overall diet quality resulted in improved outcomes for VF, fasting insulin and IR for individuals with NAFLD and T2DM. In fact, HOMA-IR reduced by a significant two-unit change in patients with T2DM in the absence of any significant weight loss. Participants with and without T2DM significantly improved adherence to the MedDiet (higher change in adherence for non-T2DM), however significant changes in body composition and biomarkers were only seen in participants with T2DM. It seems that NAFLD patients with diabetes were more sensitive to dietary change – an improvement in overall dietary quality, irrespective of diet assignment. Indeed, the development

and severity of NAFLD are closely associated with diabetes risk and T2DM.[494] The risk of NAFLD progressing to NASH, advanced fibrosis and chronic liver disease are increased with increased diabetes risk or prevalence.[563] Considering that dietary intervention remains the primary management of early diabetes, T2DM and NAFLD, an essential starting-point is early diagnosis and dietary treatment by practitioners. A routine screening process for both T2DM and NAFLD may be beneficial in treating the underlying inflammatory conditions, as identified in the responsiveness to dietary intervention observes in the present study. Moreover, with increasing rates of NAFLD and pressures on healthcare services, in terms of prioritising referrals to dietetic services, patients with diabetes and NAFLD could be put forward as a priority.

Another factor which potentially reduced scope for change in participants in the MedDiet arm was that participants with T2DM allocated to the MedDiet group were found to have significantly greater adherence to a MedDiet at baseline than those with T2DM in the LFD group. Individuals with NAFLD and T2DM who were randomised to the LFD showed the most distinguished change in body weight, VF, fasting insulin and IR. Although not statistically significant, the reduction in fasting insulin and IR in the MedDiet could be considered clinically significant and occurred in participants who did not experience weight loss. The implications of a higher number of participants diagnosed with T2DM in the LFD arm may have contributed to the effects observed of the prescribed dietary intervention. Clinical guidelines for patients who present with diabetes or pre-diabetes recommend adherence to the Australian Dietary Guidelines, which is typically a low-fat diet aiming to achieve 5-7% reduction in body weight.[564] A minimum of 6-months lifestyle intervention is recommended prior to pharmacotherapy being considered.[564] In the present study, participants who were diagnosed with diabetes and/or taking prescribed medication to control blood glucose levels were included in the cohort. It is likely that these participants would have been participating in a multidisciplinary approach, receiving diet and lifestyle advice from an APD in their respective outpatients' clinics. The additive and ongoing nutritional advice from clinical care into the clinical trial may have highlighted the importance of dietary management to participants, further enhancing adherence and improvements on clinical and metabolic outcomes.

There were a number of key differences observed between participants in the MedDiet and LFD groups at baseline, including fasting glucose, insulin and hs-CRP. Upon analysing participants randomised to each diet group, a difference (albeit small) in the distribution of participants with and without T2DM was observed between groups in that there were more participants with T2DM in the LFD group than the MedDiet group. Although participants were randomised to a diet group stratified by sex and diabetes status, the uneven distribution of individuals with T2DM is thought to have occurred due to the underpowered nature of the cohort. In the published MEDINA Study protocol,[277] a sample size calculation powered to detect a between-group difference in HOMA-IR after the 12-week intervention required 47 participants per arm (94 in total). Upon full recruitment of the predicted number of participants the distribution of individuals with T2DM and associated

biomarkers is expected to even out between diet groups, though recruitment of the full cohort was not possible in fitting with the candidate (AR) doctorate timelines.

The usefulness of inflammatory cytokines and adipokines as non-invasive surrogate markers of disease was investigated as a main outcome of this study, and it was hypothesised that the anti-inflammatory potential of the MedDiet would improve circulating serum levels of cytokine and adipokine markers. While it was expected that the MedDiet may also exert anti-inflammatory effects, lowering other key cytokines and adipokines, this effect was not observed. The present MEDINA cohort studied was small and underpowered compared to previous studies in similar populations. Based on results of similar studies, a sample size calculation was performed prior to analysis which estimated that a sample size of between 120 – 350 participants were required to see a significant change in the order of hs-CRP, TNF-α, IL-6, leptin, adiponectin and resistin. Adiponectin and resistin required the largest sample sizes, perhaps due to the sensitive nature of adipocyte secretion and detection in circulating serum. As outlined in **Chapter 2** (methods), the overarching MEDINA Trial aimed to recruit a total of 94 participants intended to detect a between-group difference in HOMA-IR (≥1.0 unit) following the 12-week intervention. There was a 1-unit change in HOMA-IR observed in the LFD group and a 2-unit change in all individuals with T2DM (regardless of diet group). This sample size was not achieved within the scope of this doctorate. The sample of participants recruited for this study was then re-calculated and based on IHL (details in methods) and a sample size of 42 was used. Clinical trials that are statistically powered to see a change in inflammatory markers and larger in sample size and duration are warranted.[565]

Limitations of this study include the small, underpowered sample size and the consequent uneven distribution of participants with diabetes between dietary intervention groups perhaps leading to a biased spread of metabolic characteristics, leading to limited scope for improvement in inflammatory markers and liver outcomes. Compared to other dietary intervention studies that reported significant changes in inflammatory markers,[380, 381, 566-569] the intervention period of 12-weeks in the present study was a somewhat short in duration As these per previous studies,[380, 381, 566-569] an intervention period of 24-weeks to one year may have allowed for additional changes in inflammatory and other blood biomarkers, body composition and liver outcomes. A greater duration would have also allowed feasibility of diets and sustainability of weight change to be observed. This study was considered a parallel groups intervention trial; however, another limitation may be that the MedDiet group were provided with food hampers at each face-to-face appointment that may have been a greater incentive to participate in the trial than the financial incentive ($20 Coles gift voucher) that the LFD participants received at the same appointments. Three participants dropped out of the trial between baseline and end-intervention timepoints and data for an additional three participants were missing at the mid-intervention time point due to missed appointments. Inconsistencies in data are expected in a human trials and because this was a relatively small cohort, outcomes are sensitive to missing data. Participants in this cohort who were classified as overweight

or obese may have potentially imprecise readings of LSM scores, as BMI is a confounder to the accuracy of Transient Elastography, Fibroscan™.[570] Another limitation of this study was the potential for under-reporting by participants in the 3-day food diaries collected. The potential for under- or over-reporting due to increased dietary awareness in self-reported questionnaires. To minimise this in this study, each participants data was thoroughly checked by a trained researcher and/or an APD during each face-to-face appointment so that any discrepancies could be discussed and resolved at that time. In order to equally compare the effect of the MedDiet and LFD, participants in each group were scheduled the same number of face-to-face dietary counselling and phone call appointments during the intervention, a study design that that reduces bias associated with clinician contact. A limitation of this design is that both groups underwent dietary intervention and therefore improvements were expected in both groups. This was not a *true* representation of a control/low-fat diet or standard care group, as the necessity for a patient to be referred to a dietitian is often missed in initial outpatient clinic visits and those who are referred to an outpatient clinical dietitian may attend an initial consult, receive some nutrition education and scheduled for follow-up every 3-6 months (on average) depending on risk status. A larger effect size is needed to observe a difference b*etween* groups, especially in a small number of participants. Also, this model of dietary intervention did not reflect a *true* control or standard care patient group.

The strengths of this study are in its study design, a well-designed randomised-controlled trial which successfully recruited a multi-ethnic population from three major metropolitan hospitals around Melbourne, Australia. This study utilised sophisticated measures for liver outcomes including ^{1}H-MRS measuring IHL and Transient Elastography, Fibroscan™ measuring LSM. A suite of inflammatory markers were assessed using circulating levels of serum and IR determined using HOMA-IR, both comprehensive measures of outcomes. The dietary recommendations for the Mediterranean diet have been previously published and adapted to intervene in a multi-ethnic population.[1] Each dietary intervention (low-fat and Mediterranean) was administered by an Accredited Practising Dietitian (APD) and dietary counselling, goal setting and follow-up were intensive.

In conclusion, the results of this study show that adherence to a MedDiet significantly improved serum adiponectin levels in the absence of weight loss. The MedDiet also led to improvements in adiposity without weight loss, indicated by the significant reduction in visceral fat in the MedDiet group. Conversely, the low-fat diet resulted in non-significant weight loss and beneficial changes in liver enzymes and insulin resistance. This change was associated with the restriction of total and saturated fat from the diet. In the pooled NAFLD cohort, overall diet quality and adherence to a MedDiet improved and a greater adherence to the MedDiet pattern was associated with lower circulating inflammatory markers hs-CRP and leptin and insulin resistance. There were additional improvements for VF, fasting insulin and IR observed in response to dietary intervention in participants who were diagnosed with diabetes and NAFLD. It is anticipated that these participants

will benefit from a ‘healthy’ dietary pattern with an emphasis on improving overall diet quality rather than weight loss or energy restricted dietary intervention. Future studies should be adequately powered and with a longer intervention to see changes in inflammatory markers hs-CRP, IL-6, TNF-α, leptin and resistin.

4.10 Linking chapter 4 and 5

The results of Chapter 4 demonstrate that improving the overall quality of the diet in patients with NAFLD is beneficial, regardless of allocation to the MedDiet or LFD intervention. Prior to the intervention being implemented, participants in both diet groups had moderate MedDiet (PREDIMED) adherence scores indicating that their diets were not of poor quality at baseline entering the intervention. At 12-weeks, a pooled cohort analysis showed that adherence to the MedDiet, as determined by PREDIMED scores, significantly increased regardless of randomisation. It is widely accepted that diets high in fruit, vegetables, wholegrains and fish and low in processed foods and sweetened beverages represent a healthy dietary pattern and these components are consistent across both the MedDiet and LFD recommendations. Participants with higher MedDiet adherence, as indicated by PREDIMED scores, had significantly lower levels of CRP, leptin and HOMA-IR than participants with lower MedDiet adherence. Moreover, results indicated that an improvement in diet quality, regardless of the type of healthy diet that was being adhered to (i.e. low-fat or Mediterranean diet) appeared to reduce visceral fat (VF) and liver enzymes (AST, ALT and GGT). At 12-weeks, the low-fat diet group did not achieve nutrient targets for fat, carbohydrate or protein and the MedDiet did not achieve nutrient targets for energy, carbohydrate, protein, total, saturated and unsaturated fats, and fibre. Despite this, improvement diet quality and nutrient density of dietary intake of all participants may have resulted in the changes observed. Despite overall improvement in diet adherence scores, anti-inflammatory effects via reduction in concentration of circulating inflammatory markers was not observed for either dietary intervention group. There are a few plausible explanations as to why the dietary interventions did not reduce inflammation in this cohort: (1) the magnitude of dietary change was not large enough between pre- and post-intervention timepoints and between diet groups; (2) the relatively *moderate* adherence to a MedDiet at baseline gave little scope for significant change; (3) the small sample size lacked statistical power. Despite this, the inflammatory potential of diets and positive dietary change (as a whole or as individual components) could inform more specific effects on circulating inflammatory markers.

Low-grade chronic inflammation, characterised by elevated circulating inflammatory cytokines and adipokines, is thought to be the most important factor in causing and maintaining metabolic imbalance which is fundamental in the progression of NAFLD toward higher risk cirrhotic states.[296] The dietary inflammatory index (DII) was derived based on food parameters and nutrients that had a direct effect on inflammatory cytokine signalling pathways systematically extracted from the literature. The cumulative effect of anti-inflammatory dietary components have the potential to lower inflammation and biochemical changes which lead to obesity, accumulation of intrahepatic lipids, fibrosis and cirrhosis. The DII differs from other diet indices, including the PREDIMED score, which assess diet patterns relating to food intake or adherence to a dietary guideline. In order to further investigate the inflammatory potential of diet quality improvement in this cohort, the

authors of the novel measurement tool the Dietary Inflammatory Index (DII) collaborated with the MEDINA team and data from the intervention was shared for calculation and analysis of DII Scores.

5 Dietary Inflammatory Index, Diet Quality and Markers of Inflammation in patients with Non-Alcoholic Fatty Liver Disease following a 12-week Dietary Intervention.

5.1 Abstract

Introduction: Inflammation is an underlying feature of the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD). Poor diet and sedentary lifestyle contribute to the accumulation of adipose tissue and secretion of inflammatory markers which promote a pro-inflammatory milieu. The Dietary Inflammatory Index (DII) is a novel tool, designed to measure the theoretical inflammatory potential of diet. A higher DII score represents a more pro-inflammatory diet which has been associated with increased inflammation and higher prevalence of cardiometabolic diseases. The **aim** of this chapter was to assess the effects of improved diet quality following adherence to a low-fat or Mediterranean diet intervention on the DII score of patients with NAFLD.

Methods: Participants with NAFLD who underwent a 12-week dietary intervention trial were included in this study. The DII score was calculated using the food intake data collected from 3-day food diaries at baseline (0-week) and end of intervention (12-weeks). Diet quality and adherence to the MedDiet was assessed using PREDIMED scores. Additionally, inflammatory markers, biochemistry, liver outcomes, anthropometry, body composition and haemodynamic markers were evaluated.

Results: Thirty-eight participants were included in this study, of whom 60% were female, mean age was 52.3 ± 12.6 years and mean BMI was 32.2 ± 6.2kg/m^2. At baseline, DII score of this NAFLD cohort was -0.06 ± 1.71 which decreased to -0.32 ± 1.86 at 12-weeks (a non-significant reduction: -0.26 ± 2.38, p =0.51). Participants with higher adherence to the MedDiet had lower (more anti-inflammatory) DII scores at 12-weeks, and a significant inverse association was observed between DII and PREDIMED score, a surrogate measure of diet quality reflecting adherence to the MedDiet. No significant association was observed between higher adherence to PREDIMED dietary components and DII, Greater anthropometric changes were observed in participants with increasing DII from baseline to end intervention (pro-inflammatory change group), whereas VF was significantly reduced in the anti-inflammatory group compared to the pro-inflammatory group.

Conclusion: Improving diet quality through healthy diet intervention improved DII score, albeit non-significantly, in individuals with NAFLD. Change in DII was not linked to a change in inflammatory markers, however a reduction in visceral fat was observed with a reduction in DII. Adherence to MedDiet principles was associated with an anti-inflammatory DII effect, which may be mediated by adiposity. Future studies should recruit larger cohorts of patients with more severe

disease phenotype to explore the impact of diet quality on DII, inflammatory markers and adiposity in NAFLD.

5.2 Introduction

Non-alcoholic fatty liver disease (NAFLD) is now recognised as the most prevalent liver disorder world-wide affecting approximately 25-30% of adults in Westernised countries.[571] NAFLD is strongly associated with metabolic disorders and type 2 diabetes mellitus (T2DM),[572] and is characterised by a chronic state of low-grade systemic inflammation which is one of the main underlying pathophysiological contributors to disease. Inflammatory markers are increasingly being investigated as therapeutic targets for the treatment of NAFLD, in addition to classic risk factors of disease such as insulin resistance (IR).[573] IR is also recognised as a key causative factor, acting as a link between metabolic abnormalities, driven by obesity and poor-quality diet.

Several studies have reported the sub-optimal dietary intake patterns of cohorts of people with NAFLD [245, 399, 574] and the association between poor diet quality with the progression from simple to severe steatosis and fibrosis been demonstrated.[575, 576] Dietary profiles known to drive the development and progression of NAFLD include an excess consumption of saturated fatty acids (SFA), fructose and cholesterol, and a low consumption of antioxidants and omega-3 (n-3) fatty acids (FAs).[246] Human dietary intervention trials traditionally focused on calorie-restriction and/or weight-loss to reduce hepatic steatosis, however, limited data is available regarding sustainability of adherence to calorie-restricted diets in the long-term for patients with NAFLD.[246] The link between NAFLD, inflammation and oxidative properties of diet are increasingly presented in the literature and dietary patterns containing these properties may assist in alleviating metabolic and liver abnormalities in the absence of weight-loss. The Mediterranean Diet (MedDiet), an evidence based anti-inflammatory diet, has been associated with improved insulin resistance (IR) and metabolic outcomes in T2DM,[577, 578] the Metabolic Syndrome (MetS) [247, 501] and NAFLD.[295]

Diet, in particular adherence to dietary patterns, is a key contributor to inflammation.[579, 580] The Dietary Inflammatory Index (DII©) is a score designed to estimate the overall inflammatory potential of one's diet.[338] It was developed following a detailed review of each diet-inflammatory marker effect reported in the literature and an algorithm based on the pro- or anti-inflammatory properties of 45 known food, nutrient or food constituent intake parameters was created.[338] Established inflammatory markers including C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are used in the DII algorithm.[338] The final DII model was 'anchored' to actual food consumption data from eleven populations world-wide and was validated in healthy participants using hs-CRP levels and DII data collected carefully through 24-hour dietary recall or food records..[339] Researchers found that the DII was able to predict the odds of elevated CRP in a healthy population (OR = 1.08; 95% CI 1.01, 1.16, P = 0.035 for data derived from 24-hr recall; and OR = 1.10; 95% CI 1.02, 1.19, P = 0.015 for data derived from 7-day dietary recall).[339]

The DII score has also been found to significantly predict higher levels of circulating IL-6 and TNF-α.[340, 341]

The MedDiet includes a range of individual foods, nutrients and non-nutritive components that exert protective and/or preventative benefits to health.[246] Anti-inflammatory effects of the main components of the MedDiet are well established in the literature,[581] though recent research in the field has focused on observing the synergistic effects of the whole MedDiet rather than analysing individual components of the dietary pattern.[582] By nature of its composition, the MedDiet is likely to have an anti-inflammatory DII score and greater adherence to a MedDiet will likely lead to an anti-inflammatory change in DII. A prospective cohort study reported high ('pro-inflammatory') DII scores were positively associated with risk of lung cancer and inversely associated with a Mediterranean Diet score.[583] In an obese Mediterranean population, researchers found that a more pro-inflammatory diet (according to DII score) was associated with increased risk of obesity and annual weight gain.[584] Only few published intervention trials have analysed the effect of dietary interventions on DII, of which; one study reported short-term improvements whilst following a vegan or vegetarian diet,[585] another reported moderate, long-term improvements with a low-fat diet,[586] and another found significant improvements following a Mediterranean Diet and no improvements following a low-fat diet (LFD).[365]

Mayr et al. (2018) administered the same dietary interventions (MedDiet vs. LFD) as the present (MEDINA) study to individuals diagnosed with coronary heart disease (CHD). The DII score of the MedDiet and LFD interventions that were administered in the present study (overall and individual food/nutrient parameters) have been calculated and published.[365] Both diets had an anti-inflammatory DII score, however the MedDiet meal plan had a noticeably greater anti-inflammatory DII score than that of the LFD (-4.55 vs. -0.33, respectively). The meal plans of the MedDiet and LFD contained some similarities for intake of DII nutrient parameters however they also contained some key differences. The MedDiet is higher in total fat content, which is classified as a pro-inflammatory parameter, but also higher in unsaturated fatty acids MUFA and PUFA, n-3 and -6, fibre, alcohol, vitamin A, D and E, flavonoids, garlic, onion, rosemary and thyme/oregano, which are all anti-inflammatory DII parameters.[365] The relatively high intake of herbs and spices in the MedDiet contain biologically active anti-inflammatory compounds[587] expected to contribute to its low DII score.[588]

Few studies have examined the association between DII and liver disease, though a pro-inflammatory DII score has been linked with an unfavourable body mass, higher liver enzymes, fatty liver index (FLI) score, and therefore likelihood of fatty liver, in a generally healthy population[344] and in overweight and obese individuals in the PREDIMED cohort.[343] It should be noted that these results are from large studies in which not all participants were diagnosed with NAFLD and non-specific indices, such as FLI, were used to assess hepatic outcomes.[343, 344] A number of studies have been conducted to test associations between the MetS and DII, however

results remain inconclusive. In a cohort study a higher DII score was associated with a higher risk of MetS, blood pressure and triglycerides.[589] In contrast, four cross-sectional studies[590-593] and two cohort studies[594, 595] detected no association in their populations. The association between the DII and liver, metabolic and inflammatory outcomes has not been published in a NAFLD population, nor the effect of dietary intervention on DII in this context.

Due to the small sample size of participants, datasets from both dietary intervention groups were pooled and their exposure and outcome data analysed as one group for this chapter. The motivation to combine both diet groups was to increase the sample size and statistical power to detect treatment effects, which may increase the precision of the estimation.[596] Pooling the datasets for analysis was deemed appropriate due to the similar improvement in diet quality and adherence to MedDiet that was observed within the groups in Chapter 4 of this doctoral thesis.

Therefore, the primary aim of this chapter was to assess the effects of improved diet quality (following adherence to a low-fat or Mediterranean diet intervention) on the DII score of patients with NAFLD. The secondary aims of this chapter were to:

(i) assess whether improvements in diet quality, as assessed by higher MedDiet adherence score, lead to concomitant improvements in DII score following dietary intervention

(ii) determine which nutrients, food groups or MedDiet components were associated with a more favourable DII score following dietary intervention

(iii) elucidate whether a dietary-induced reduction in DII score was associated with improvements in inflammatory, hepatic and metabolic-risk markers, and anthropometry and body composition measures.

It was hypothesised that patients with NAFLD who underwent a 12-week dietary intervention and improved diet quality regardless of randomisation, would experience a reduction (improvement) in DII score, and would improve serum inflammatory markers.

5.3 Methods

5.3.1 Study Design and Participants

The MEDINA study is a 12-week parallel, randomised controlled trial in patients with ultrasound or biopsy proven NAFLD and NASH.[345] A full description of the study design, recruitment sites and process, eligibility criteria and timeline of appointments is presented in **Chapter 2** of this thesis. Data from the baseline (0-week) and end intervention (12-week) timepoints was used for this analysis.

Participants in this study were recruited from liver outpatient clinics of three major metropolitan hospitals around Melbourne, Australia. These included the Alfred Hospital, Eastern Health and the Royal Melbourne Hospital. Briefly, eligible patients were adults (>18 years) who were diagnosed with NAFLD and/or NASH via ultrasound and/or biopsy within 12-months of recruitment, had a body mass index (BMI) of 20-40 kg/m^2 and elevated serum alanine aminotransferase (ALT) level (>20U/L for females and >30U/L or males). Eligible participants were free of any other form of liver disease or current or past cardiovascular, cerebrovascular or peripheral vascular diseases, were English-speaking, did not consume more than 140g of alcohol per week, had not gained or lost more than 5kg body weight within 3-months of recruitment and were not adhering to any commercial diet or consuming hepatotoxic medication, fish or krill oil, or vitamin E, vitamin C or high dose vitamin D.

5.3.2 Dietary Interventions

A full description of the prescribed diets, dietary counselling techniques and recommendations and timeline of participant follow up which was conducted by an Accredited Practicing Dietitian is described in **Chapter 2** (**Sections 2.6 and 2.7**) of this thesis. Briefly, participants were randomised to either a Mediterranean Diet (MedDiet) or Low-Fat Diet (LFD) for a 12-week intervention period. Both intervention groups received an equal amount of face-to-face and phone call consultation time with the APD and both diets were delivered in an *ad-libitum* approach. Physical activity recommendations were not provided to any participants and weight loss was not a main outcome of the MEDINA study.

The MedDiet was based on a traditional Cretan Mediterranean Diet,[279, 345] recommendations for which are described in George et al. (2018).[1] The approximate macro and micronutrient composition of the MedDiet was 44% fat (>50% monounsaturated), 36% carbohydrate and 17–20% protein, and up to 5% alcohol. A model diet (2-week meal plan) was created for participants to follow which contained key components of the MedDiet, including; high consumption of plant-based foods, fruits, vegetables and wholegrains, moderate consumption of legumes, nuts and oily fish, moderate consumption of fermented dairy products and white/game meats, and decreased consumption of red meat and sweetened or processed foods. The main source of culinary fat was extra-virgin olive oil (EVOO). The meal plan provided was equivalent to approximately 9,400kJ of total energy per day.

The dietary recommendations provided as part of the LFD intervention were based on the Australian Dietary Guidelines and Heart Foundation recommendations,[101, 356] and the Australian Guide to Healthy Eating was used for education around low-fat cooking methods, portion sizes and low-fat options. The macronutrient composition of the LFD was approximately 30% fat, 50% carbohydrate and 20% protein.

The present chapter was not designed to compare the effect of the MedDiet versus LFD intervention on DII scores because the change in macro and micronutrients reported in Chapter 4 dietary intake analyses were deemed to be not too diverging to observe differences in the effects in DII between the diets. It was anticipated that dietary changes between and within diet groups were too small to significantly influence DII. Number of participants in each diet group was also a limiting factor and were considered too small to have sufficient statistical power to detect a change in DII. Both dietary interventions are classified as 'healthy' diets and can influence positive improvements in overall diet quality. Hence, the effect of overall improvement in diet quality following the prescribed diet recommendations and its impact on DII score was analysed in this chapter. To achieve this, the entire cohort of participants with NAFLD were pooled (n=42) and the subsequent DII analysis included all participants regardless of diet group allocation.

5.3.3 Primary Outcome Measures – Dietary Inflammatory Index (DII®)

The development and validation of the DII has been previously published.[338, 339] The DII is a score designed to quantify the overall inflammatory potential of a diet. Researchers conducted a literature review of peer-reviewed articles from 1950 to 2010, which investigated the role of whole foods and dietary constituents on specific inflammatory markers: IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP.[337, 338] The search retrieved 1943 articles and forty five food parameters were linked to chosen inflammatory markers. Each article was assigned one of three possible values based on the effect of the food parameter on the inflammatory markers a pro-inflammatory effect (+1) where IL-1β, IL-6, TNF-α or CRP significantly increased, or IL-4 or IL-10 significantly decreased; an anti-inflammatory effect (-1) where IL-1β,IL-6, TNF-α or CRP significantly decreased, or IL-4 or IL-10 significantly decreased; or no change (0) if the food did not produce any significant change in inflammatory marker. For studies which may have shown differential effects of one food parameter on inflammatory markers (i.e. a food parameter increased one inflammatory marker whilst decreasing another; both pro- and anti-inflammatory changes), the article would receive +1 for the pro-inflammatory effect and -1 for the anti-inflammatory effect.[338] The study type, design and total number of research articles determined the weighting allocated to each food parameter. To calculate a parameter-specific inflammatory effect score, the weighted score of pro- and anti-inflammatory articles were then divided by the total weighted number of articles and the anti-inflammatory fraction was subtracted from the pro-inflammatory fraction. The median total weighted number of articles across all of the food parameters was 236, therefore this value was chosen as a cut-off point to indicate an optimally robust pool of literature. If a food parameter had a weighted number of articles ≥236 they were assigned the full value of the score and if the food parameter had a weighted number of articles <236 then the number was divided by 236 to find a fraction, then multiplied by the food parameter-specific raw inflammatory score of the food parameter, which resulted in the food parameter-specific overall inflammatory effect score. Dietary data were then linked to a database representative of food consumption from 11 populations world-wide that provided a daily

mean and standard deviation for each food parameter used to derive the score. A z-score was obtained for each parameter which was converted to a percentile (minimising skewing or the effect of outliers) and centred on zero (0) by doubling the value and subtracting 1.[338] This centred percentile score for each individual food intake parameter was then multiplied by its inflammatory effect score (score derived from the literature review) to form an overall DII score for an individual.

In the present study, DII was assessed for each participant at baseline and at 12-weeks based on 28 of the 45 food parameters available for the MEDINA cohort. The food parameters used to calculate DII in this study were: energy, carbohydrate, total fat, saturated fat, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), omega 3, omega 6 (linoleic acid only), trans fat, protein, alcohol, vitamin B12, vitamin B6, vitamin A, vitamin C, vitamin E, caffeine, cholesterol, fibre, beta carotene, folic acid, iron, magnesium, niacin, riboflavin, selenium, thiamine and zinc. Food parameters that were not available for the MEDINA cohort were: eugenol, garlic, ginger, saffron, turmeric, vitamin D, green/black tea, flavan-3-ol, flavones, flavanols, flavanones, anthocyanidins, isoflavones, pepper, thyme, oregano and rosemary. The food parameters not available for analysis had overall inflammatory effect scores ranging between -0.013 and -0.785, reflecting 'anti-inflammatory' scores. Therefore, the anti-inflammatory scope of the overall DII score may be lessened due to the missing food parameters in this group. All dietary intake parameters were extracted from FoodWorks9 nutrient and food group analysis data collected via 3-day food diaries (detailed above).

5.3.4 Secondary Outcome Measures

5.3.4.1 Dietary intake

Participant's dietary intake was recorded using a 3-day food diary, completed prior to the baseline and 12-week appointments. Household measures were used to record foods consumed on two weekdays and one weekend day, specifying the type, brand, quantity and cooking methods of food and beverage items. The diaries were checked, and missing details clarified by the dietitian at each appointment. Each day recorded should have represented a 'normal' daily intake for the participant and if it did not (i.e., was abnormal) then the data was not used. Participant food diaries were entered and analysed in FoodWorks9™ using the most up-to-date *AUSNUT 2013, AusBrands 2015* and *AusFoods 2015* databases. Macronutrient, micronutrient and food group analysis was exported from FoodWorks9™ and further analysis was conducted in SPSS® statistical package version 25 (IBM Corp, Released 2017).

5.3.4.2 Mediterranean Diet Adherence

Dietary adherence was measured using a 14-item Mediterranean Diet scale which was generated and validated by the Prevención con Dieta Mediterránea (PREDIMED) study.[364] This checklist includes key dietary components and desirable number of food serves that are considered to be

staples in the MedDiet (for example, olive oil used as primary fat source and ≥4 tablespoons consumed per day). If the participant was adhering to a component, they would be allocated one point for the criterion to which they were adhering. A higher MedDiet score indicates greater adherence to a traditional MedDiet pattern and a total score of ≥9 out of 14 is considered an acceptable level of adherence.[597] An example of the PREDIMED checklist is provided in **Appendix 6.2**. Participants completed PREDIMED checklists prior to the baseline and 12-week appointments, and during the appointment they were checked by the dietitian to correct any errors or misinterpretation, as well as to cross-check the checklist responses against dietary intake from 3-day food diaries.

5.3.4.3 Inflammatory Markers

A fasting blood sample was taken from participants during the baseline and 12-week appointments, the samples were collected in a Serum Separator Tube (SST®), immediately centrifuged at 2.4 RPM for 10 minutes and aliquots were stored at −80 °C until a full-batch for each timepoint had been collected. Serum high-sensitivity (hs)-CRP was analysed by the Alfred Hospital Pathology Laboratory in Prahan, Melbourne. Serum adiponectin and TNF-α were analysed using Milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA) as previously described in Gabel et al. (2016).[357] The analysis was conducted by a trained MEDINA researcher (AR) and collaborators at Deakin University, Melbourne, Australia. The assay was performed as per manufacturer instructions and samples were run in duplicate.

5.3.4.4 Liver Outcomes

Intrahepatic lipid (IHL) content of the liver was measured using proton magnetic resonance spectroscopy (^{1}H-MRS), the gold-standard test to quantify and monitor changes in IHL percentage (%). The test was performed by a qualified radiographer at the Baker Heart and Diabetes Institute located at the Alfred Hospital, Melbourne, Australia. Each participant also underwent Transient Elastography (FibroScan®) to measure liver stiffness and change in fibrosis over the study duration carried out by a Hepatologist.

5.3.4.5 Biochemistry

Participants arrived fasted to each appointment and trained phlebotomy staff at the Alfred Hospital Pathology Clinic collected and prepared blood samples according to standard protocol. Full details of pathology protocol and biomarkers analysed are presented in **Chapter 2** (**Section 2.8.3.2** and **Table 2.4**).

5.3.4.6 Anthropometry, Body Composition and Haemodynamic Markers

Weight, height, waist circumference and blood pressure measures were taken in duplicate by a trained MEDINA researcher (EG/AR) at each appointment. Measurements were taken using

standard procedures and the average of two values was used as the final measure. Bioelectrical impedance analysis was used to measure body composition and body mass index at the Alfred Hospital Prahan, Melbourne, during each appointment. Standard procedures for the Seca© machine were followed by a trained researcher.

5.3.5 Statistical Analysis

For the purpose of this analysis, all variables for patients with NAFLD participating in the MEDINA study were pooled and measurements analysed regardless of treatment received in the allocated dietary intervention arm. The results of Chapter 4 of this thesis were used as the rationale for combining MedDiet and LFD groups for analysis. Specifically, both diet groups improved similar areas of their diet which was believed to have occurred due to a cross-over of "healthy" dietary recommendations. In order to investigate the full impact that these dietary changes had on DII score, the cohort was not split by diet group. The present analysis was designed as an exploratory study of diet and DII in a relatively small NAFLD cohort over a 12-week dietary intervention period which has not previously been investigated in this population, therefore no power calculation was performed using DII as the main outcome. Mean change values were calculated from baseline (0-weeks) and end-intervention (12-weeks) timepoints. At the end intervention timepoint, data was available for n=38 participants and missing data was excluded (i.e., 38 participants used in all analyses, complete case analyses). No attempt was made to replace missing values because this analysis aimed to assess dietary changes following the intervention period and non-completers do not represent this change.

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 25 (IBM Corp, Released 2017). Data are presented as n (%) and mean ± SD as appropriate. Normality of continuous variables were assessed using Kolmogorov-Smirnov statistic. Based on normality, an independent samples t-test or Mann-Whitney U test were used to compare pre-post differences in continuous variables. Differences were considered statistically significant at $p < 0.05$. In this study, a DII score of zero was considered neutral, less than zero (<0) represented anti-inflammatory potential of a food parameter or an anti-inflammatory change in DII score and a DII score above zero (>0) represented pro-inflammatory potential of a food parameter or a pro-inflammatory change in DII score. Change in DII score was calculated for each participant based on the mean change in DII score between baseline and end-intervention timepoints; an increase in DII score between timepoints was classified as a pro-inflammatory change and a decrease in DII score between timepoints was classified as an anti-inflammatory change. The relationship between change in dietary intake with DII score was assessed by means of partial correlation tests for parametric and non-parametric variables. Partial correlations were used to determine the association between inflammatory markers, liver outcomes, biochemical and anthropometric variables with DII

score at end intervention, controlling for change in energy (kJ) intake. Correlation p-values are two-tailed and significant at the p <0.05 level. R-value cut offs were: 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.[598] All values of serum hs-CRP that were >10mg/L were excluded from analysis as this is reflective of acute inflammation rather than chronic inflammation.[156] This excluded n=3 participants at baseline and n=6 participants at end-intervention.

5.4 Results

A total of 42 participants with NAFLD were enrolled in the MEDINA study at baseline, three of whom withdrew (due to family or medial reasons) prior to completing the intervention (7% attrition) and a further one participant did not have dietary intake data available at the end intervention timepoint. Therefore, a total of 38 participants were included in this study. Of these participants, 60% were female, the mean age of participants was 52.3 ± 12.6 years and mean BMI was 32.2 ± 6.2kg/m^2.

5.4.1 DII score of the NAFLD cohort

The baseline DII score of this NAFLD cohort was -0.06 ± 1.71 which decreased to -0.32 ± 1.86 after a 12-week dietary intervention period. At baseline, the DII score ranged between -2.88 and 3.52 and at the end of intervention ranged between -3.40 and 3.51. In the entire NAFLD cohort, DII from baseline to end intervention reduced non-significantly (-0.26 ± 2.38, p =0.51), a change that represents a more anti-inflammatory shift.

In the following results sections, DII score was analysed both as a continuous variable and as a dichotomous variable, categorised based on the cut-off value of zero (0). When DII was dichotomised, participants who experienced a 'pro-inflammatory change' (or increase (+)) in DII score were grouped and those who experienced an 'anti-inflammatory change' (or reduction (-)) in DII score were grouped together. Three-day food dairies were used to derive DII score and group participants who experienced a change in DII score of 0.00 to 5.96 into a 'pro-inflammatory change' group (n=17) and those who experienced a change in DII score of -4.34 to <0.00 into an 'anti-inflammatory change' group (n=21).

Figure 5.1 illustrates the study flow of participants who moved between anti- and pro-inflammatory DII groups, classified according to DII score below and above zero. Diet group allocation of participants was included in this figure to assist the identification and interpretation of the potential impact of dietary intervention. Twenty-three participants were classified as having an anti-inflammatory diet (DII <0.00) at baseline, and the spread of participants in MedDiet and LFD groups were even. The proportion of participants adhering to a more anti-inflammatory diet at baseline (n=23, 55%) did not change significantly at 12-weeks (n=21, 55%) (p >0.05). There were two dropouts, both from the LFD arm, 13 participants remained consuming an anti-inflammatory

diet and eight participants who were consuming a pro-inflammatory diet at baseline (DII >0.00) were classified as consuming an anti-inflammatory diet at 12-weeks. The proportion of participants in the MedDiet versus LFD groups who were consuming anti-inflammatory diets at baseline remained the same as at the end of intervention. Nineteen participants were consuming a pro-inflammatory diet (DII >0.00) at baseline, of whom 11 participants were in the LFD group and eight participants were in the MedDiet group. One participant from each diet group dropped out of the study, nine participants remained in the pro-inflammatory group and eight participants (four from each diet group) changed from consuming an anti-inflammatory diet to a pro-inflammatory diet by end intervention. The number of participants consuming a pro-inflammatory diet at end intervention who were allocated to the MedDiet group remained the same as baseline (n=8), whereas LFD participants in this group decreased (n=2).

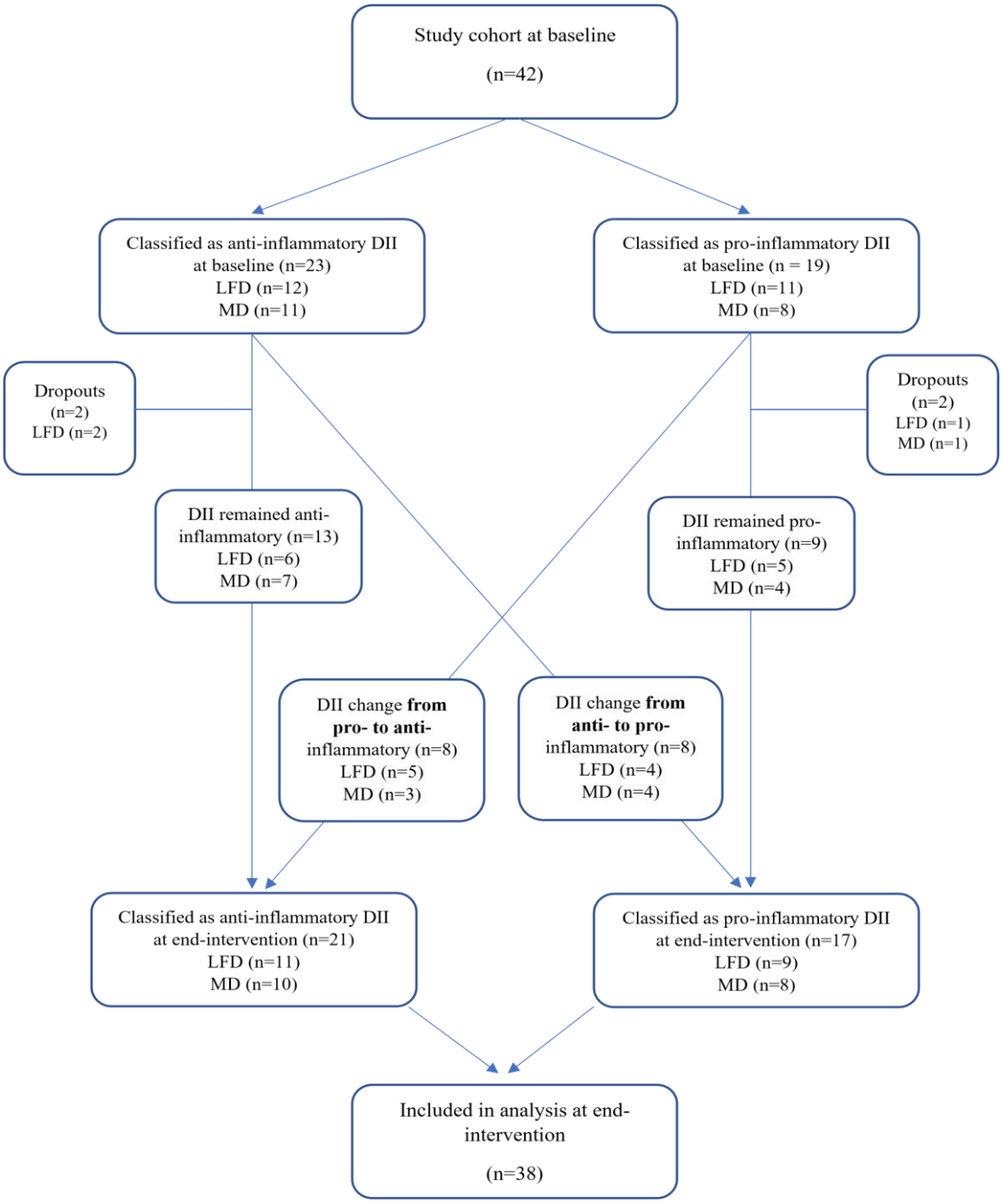


Figure 5.1. Study flow diagram of the pooled cohort of MEDINA participants, distribution is categorised by anti-inflammatory (<0.00) versus pro-inflammatory (>0.00) DII score at study timepoints and comparison of diet study groups.

n=4 dropouts. Abbreviations: DII, Dietary Inflammatory Index; MD, Mediterranean Diet; LFD, Low-Fat Diet.

At 12-weeks, mean DII score of the anti-inflammatory group was -1.19 ± 1.37 and mean DII score of the pro-inflammatory group was 0.76 ± 1.86 (p =0.001, between groups).

Mean change in DII score in anti- and pro-inflammatory groups is presented in **Table 1**. Following the dietary intervention there was a non-significant reduction in the DII score of participants in the anti-inflammatory group and a non-significant increase in DII score of those in the pro-inflammatory DII group.

5.4.2 DII and Dietary Intake in a NAFLD cohort

The nutrient and food group intake of individuals classified into groups of pro-inflammatory (positive) or anti-inflammatory (negative) change in DII score are presented in **Table 5.1**.

Consumption of protein, PUFAs, omega-3, linoleic acid, fibre, vitamin C, vitamin E, beta-carotene, magnesium, potassium, serves of fruits, vegetables and oil equivalents was significantly (p <0.05) higher in participants with a reduction (anti-inflammatory change) in DII score compared to participants with a pro-inflammatory change following an intervention to improve overall diet quality.

Participants who experienced an increase in DII score from baseline to 12-weeks (pro-inflammatory change) reduced their total energy and carbohydrate intake more significantly (p <0.05) than participants who experienced a decrease in DII score (anti-inflammatory change).

Table 5.1. Baseline to 12-week change in PREDIMED scores for MedDiet adherence and nutrient and food group intake across change in DII groups

Change Variable	***Change in DII***				**p**[a]
	Anti-inflammatory (-4.4 to 0.0) n=21		**Pro-inflammatory** (0.0 to 6.0) n=17		
	mean	SD	mean	SD	
DII Score					
DII	-1.92	1.29	1.80	1.71	<0.001*
Nutrients					
Energy (kJ)	-202.8	2216.8	-2135.8	2518.8	0.02*
Carbohydrate (g)	-17.7	55.4	-73.8	81.0	0.02*
Protein (g)	5.0	35.4	-20.7	28.0	0.02*
Fat (g)	-1.4	31.9	-14.7	35.2	0.23
Saturated fats (g)	-7.7	13.7	-6.0	11.9	0.68
MUFA (g)	1.9	15.9	-2.6	20.2	0.45
PUFA (g)	4.4	6.4	-5.2	9.0	0.001*
Omega-3 (g)	1.0	1.2	-0.6	1.5	0.001*
Linoleic Acid (g)	3.4	6.1	-4.5	7.8	0.001*
Dietary Fibre (g)	6.3	7.5	-7.8	9.5	<0.001*
Alcohol (g)	-0.1	6.8	1.7	5.7	0.39
Vitamin C (mg)	71.4	90.9	-110.4	185.8	<0.001*
Vitamin E (mg)	3.6	5.0	-2.9	9.7	0.011*

Folate (µg)	-46.3	265.1	-162.1	207.5	0.15
Beta-carotene (µg)	797.4	4228.7	-2666.3	3543.3	0.011*
Magnesium (mg)	47.2	90.0	-95.3	137.1	<0.001*
Potassium (mg)	443.3	786.6	-1039.3	1033.9	<0.001*
Sodium (mg)	-180.7	937.5	-359.0	1111.3	0.60
Food groups					
Fruit /d	0.3	1.3	-0.7	1.3	0.015*
Vegetables /d	1.5	2.5	-2.9	2.8	<0.001*
Wholegrain cereals /d	0.7	1.3	0.9	2.6	0.77
Refined cereals /d	-1.6	3.9	-1.9	2.4	0.72
Dairy /d	-0.2	0.6	-0.7	1.5	0.19
Red Meats /d	-0.2	0.7	-0.4	1.4	0.56
Seafood serves /d	0.2	0.7	0.3	0.7	0.53
Legumes /d	0.0	0.6	-0.1	0.5	0.48
Nuts /d	0.1	0.9	-0.2	1.2	0.44
Oil equivalents (serve) /d	3.2	4.1	-1.0	6.6	0.021*
Alcohol beverages /d	0.0	0.7	0.2	0.6	0.39

Participants split into groups of 'pro-inflammatory' (above 0.00) or 'anti-inflammatory' (below 0.00) change in DII over 12-weeks. Negative values represent an anti-inflammatory change in DII score and positive values represent a pro-inflammatory change in DII score. Collinearity may be present in all variables found to be significantly different, except potassium, due to their inclusion in the calculation of the DII. Abbreviations: DII, Dietary inflammatory index; Energy-Density DII (E-DII); SD, standard deviation; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. P[a] p-value for comparing mean change values between pro- vs anti-inflammatory groups; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. * indicates significance ($p < 0.05$).

Partial correlations were carried out to assess the association between the variable change in DII score and nutrient and food group intake variables, controlling for baseline nutrient intake or food group variable in each analysis (presented in **Table 5.2**). A positive change in DII score was reflective of a more pro-inflammatory DII. A moderate, inverse and significant ($p < 0.05$) correlation was observed between change in DII score and the following nutrient and food variables: protein, omega-3, vitamin C, folate, beta carotene, and servings of fruit and nuts. A strong, inverse and significant ($p < 0.05$) correlation was observed between change in DII score and the following variables: PUFA, linoleic acid, dietary fibre, vitamin E, magnesium, potassium, and serves of vegetables and oil equivalents. Change in energy intake from baseline to 12-weeks was also found to have a moderate, inverse and significant correlation with change in DII ($r = -0.45$, $p = 0.005$).

Table 5.2. Partial correlations between change in DII and nutrient and food group (change) variables, values adjusted for baseline nutrient/food group variable

Change Variable	**Correlation Coefficient**
Nutrients	**r (p)**
Energy (kJ)	-0.45 (0.005*)
Carbohydrate (g)	-0.31 (0.064)
Protein (g)	-0.42 (0.01*)
Fat (g)	-0.29 (0.077)
Saturated fats (g)	0.07 (0.683)

MUFA (g)	-0.28 (0.099)
PUFA (g)	-0.53 (0.001*)
Omega-3 (g)	-0.37 (0.023*)
Linoleic Acid (g)	-0.51 (0.001*)
Dietary Fibre (g)	-0.74 (<0.001*)
Alcohol (g)	-0.04 (0.798)
Vitamin C (mg)	-0.48 (0.003*)
Vitamin E (mg)	-0.58 (<0.001*)
Folate (µg)	-0.40 (0.013*)
Beta-carotene (µg)	-0.45 (0.005*)
Magnesium (mg)	-0.69 (<0.001*)
Potassium (mg)	-0.75 (<0.001*)
Sodium (mg)	-0.22 (0.201)
Food groups	
Fruit /d	-0.38 (0.019*)
Vegetables /d	-0.60 (<0.001*)
Wholegrain cereals /d	-0.05 (0.785)
Refined cereals /d	0.05 (0.761)
Dairy /d	-0.10 (0.568)
Red Meats /d	0.02 (0.917)
Seafood serves /d	0.05 (0.764)
Legumes /d	-0.07 (0.671)
Nuts /d	-0.33 (0.046*)
Oil equivalents (serve) /d	-0.57 (<0.001*)
Alcohol beverages /d	-0.06 (0.746)

Partial correlations between change in dietary intake variable and change in DII score, controlling for baseline variable value. * Correlation is significant at the 0.05 level (2-tailed). R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.

5.4.3 DII and Diet Quality (Adherence to the MedDiet)

There was no association between the DII score and PREDIMED score at baseline (r =0.06, p =0.720). At end intervention, a moderate, inverse and significant correlation was observed between the DII score and PREDIMED score (r = -0.32, p =0.048).

The pooled cohort of participants were divided into two groups based on adherence to the MedDiet, as determined by PREDIMED score. A score of 0 – 7 represented low to moderate adherence to the MedDiet, and a score of 8 – 14 represented moderate to high adherence to the MedDiet. Mean DII score and change in DII score across the groups was calculated to determine the impact of MedDiet adherence on DII score (presented in **Table 5.3**).

At baseline, 83% of participants were classified as low to moderately adhering to the MedDiet with a mean DII score of -0.10 ± 1.81. At 12-weeks, 46% of participants remained in the low to moderate adherence group and had a mean DII score of 0.39 ± 2.01. The number of participants classified as low to moderately adhering to the MedDiet decreased from baseline to 12-weeks (i.e., shifted out

of this group, increasing their MedDiet score), however counterintuitively their DII score increased (became more pro-inflammatory) from baseline to 12-weeks. A lower proportion of participants began the study with a moderate to high adherence score (17%) and these participants had a mean DII score of 0.08 ± 1.35. At 12-weeks, participants in the moderate to high MedDiet adherence group increased to 54% and their mean DII decreased non-significantly to -0.95 ± 1.50 (p =0.117).

Table 5.3. Levels of dietary adherence (based on the PREDIMED score) at baseline and end intervention timepoints and associated mean DII score

Level of Adherence to MedDiet	Baseline		End Int		p-value	Change	
	n (%)	DII Score	n (%)	DII Score		%	DII Score
Low to Moderate (Score 0 - 7)	34 (83)	-0.10 ± 1.81	18 (46)	0.39 ± 2.01	0.394	-37	0.30 ± 3.23
Moderate to High (Score 8 - 14)	7 (17)	0.08 ± 1.35	21 (54)	-0.95 ± 1.50	0.117	+37	-0.22 ± 1.31

Participants were categorised as low to moderately or moderately to high adhering to a MedDiet pattern based on PREDIMED score at each timepoint. P-value compares mean scores within groups across timepoints; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data, significance ($p < 0.05$). Abbreviations: DII, Dietary inflammatory index; n, number of participants; %, percentage frequency of participants.

5.4.4 Components of a MedDiet and DII

Participants were classified as 'adhering' or 'not adhering' to individual components of the PREDIMED checklist based on their responses at the baseline and 12-week timepoints, and corresponding DII scores were analysed and presented in **Table 5.4 and Figure 5.2**. No significant differences were observed between groups – likely due to the lack of statistical power and limited number of participants – however, a number of trends were identified. As expected, participants who were adhering to the following components of the PREDIMED checklist experienced a larger reduction in DII score (more anti-inflammatory/favourable change) compared to participants who were not adhering to the component; EVOO consumption and EVOO quantity, vegetable intake, red meat intake, dairy intake, carbonate beverage intake, fish intake and sweets.

Conversely, participants who did not meet the PREDIMED recommendations for the consumption of fruit, legumes, nuts, and preferentially choosing white meat and adhering to the use of the sofrito method experienced a larger reduction in DII score (anti-inflammatory change) than participants who were consuming the recommended serves. In order to better understand these results, the mean DII score of participants adhering to each PREDIMED component before and after dietary intervention are reported in **Table 5.5**. Although an increase in DII score was observed for participants adhering to the recommendation of ≥3 serves of fruit per day, mean DII at baseline and end-intervention were anti-inflammatory scores (-0.81 ± 1.9 and -0.66 ± 1.5, respectively) compared to participants who were not consuming the recommended serves (baseline: 0.13 ± 1.7

and end int: -0.21 ± 1.9). Whilst non-adherers to this MedDiet component experienced a greater change in DII score over 12-weeks, adherers had a more anti-inflammatory score at both 0- and 12-week time points. Similarly, participants who adhered to the recommendations for intake of nuts had anti-inflammatory DII scores at 0- and 12-weeks (-0.4 ± 1.6 and -0.45 ± 1.8, respectively). Participants not meeting the recommendation (≥1 serve nuts per day) reduced their DII score from 0.70 ± 1.9 to 0.02 ± 2.2 which elicited a greater reduction in DII than adherers to this component, though DII remained pro-inflammatory at both time points. The majority of participants (n=33) were preferentially consuming white meat by the end of intervention and their DII score remained lower than those who were not adhering to this component (-0.14 ± 1.6 to -0.34 ± 1.8 vs 0.28 ± 2.7 to -0.18 ± 2.6, respectively). Consumption of legumes and use of sofrito cooking method was not well adhered to in this group and interestingly, non-adherers had lower DII scores. These differences may be due to the unbalanced number of participants in each group, rather than magnitude diet or DII changes. None of the changes in DII score for individual PREDIMED components were significant (p >0.05) and presented above are trends only. Only one individual was consuming ≥7 glasses of wine per week the final component of the PREDIMED score therefore associations with DII cannot be determined.

Table 5.4. Mean change in DII of participants adhering and not adhering to individual components of the PREDIMED Checklist at 12-weeks

	Adherers			**Non-adherers**			
PREDIMED Component	n	Change in DII		n	Change in DII		p value
		mean	SD		mean	SD	
EVOO	25	-0.30	2.17	13	-0.18	2.85	0.883
EVOO Quantity	10	-0.29	1.93	28	-0.18	2.56	0.910
Vegetables	27	-0.61	2.20	11	0.42	2.66	0.208
Fruit	9	0.15	1.40	29	-0.39	2.62	0.560
Red Meat	25	-0.34	2.10	13	-0.10	2.95	0.776
Dairy	30	-0.36	2.21	8	0.11	3.11	0.624
Carb. Beverages	33	-0.27	2.39	5	-0.17	2.64	0.928
Wine	1	0.96		37	-0.29	2.41	0.611
Legumes	9	0.25	2.47	29	-0.42	2.38	0.474
Fish	22	-0.63	1.93	16	0.25	2.88	0.269
Sweets	24	-0.35	2.28	14	-0.11	2.63	0.767
Nuts	27	-0.09	2.05	11	-0.68	3.13	0.492
White Meat	33	-0.23	2.19	5	-0.46	3.77	0.847
Sofrito	17	0.53	2.12	21	-0.90	2.44	0.063

Participants were distributed into groups of adherers ('yes' response on checklist) or non-adherers ('no' response on checklist) based on responses to each PREDIMED checklist criteria at end-intervention. Negative values represent an anti-inflammatory change in DII score and positive values represent a pro-inflammatory change in DII score. Abbreviations: DII, Dietary inflammatory index; SD, standard deviation; PREDIMED, Prevención con Dieta Mediterránea; EVOO, extra-virgin olive oil. P-value compares mean scores across groups; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. * indicates significance (p <0.05).

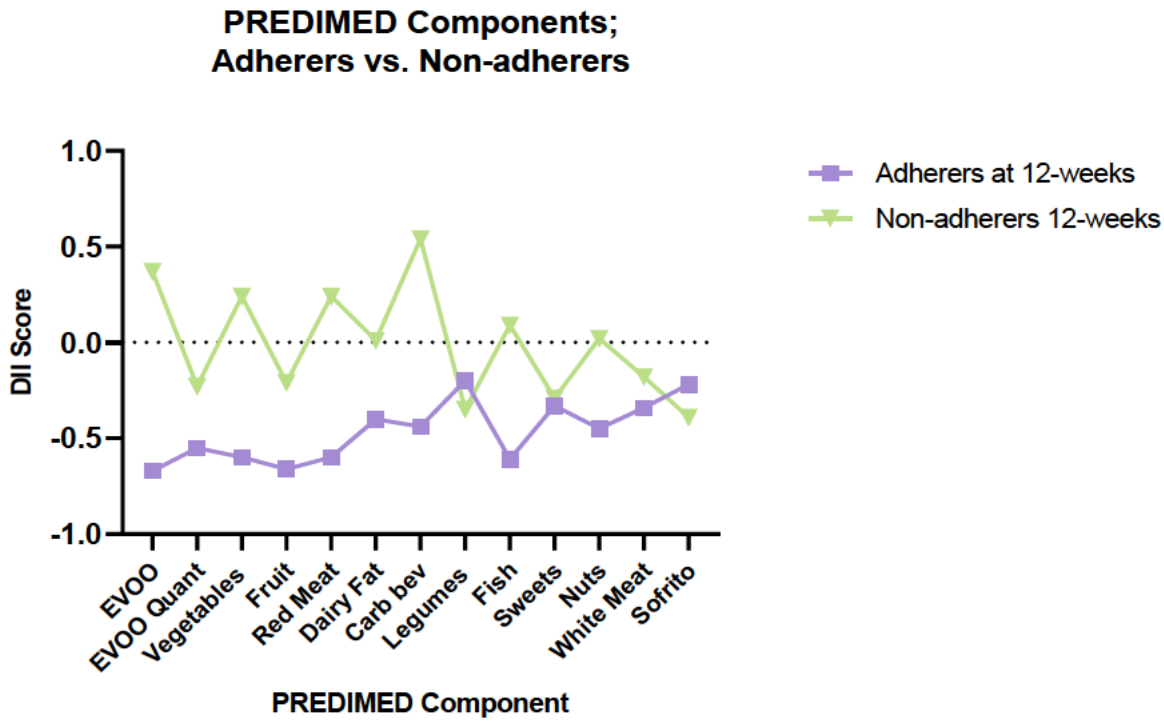


Figure 5.2. The DII score of participants classified as adhering versus not adhering to individual components of a MedDiet, based on the PREDIMED checklist.

Only n=1 participant was consuming wine in this group, therefore this component of the PREDIMED score was removed to avoid skewing of data.

Table 5.5. Dietary Inflammatory Index scores of participants adhering versus not adhering to individual components of the PREDIMED Checklist baseline and 12-weeks

PREDIMED Component	Adherers Baseline			Adherers 12-weeks			p-value	Non-adherers Baseline			Non-adherers 12-weeks			p-value
	n	mean	SD	n	mean	SD		n	mean	SD	n	mean	SD	
EVOO	17	-0.37	1.7	25	-0.67	1.9	0.599	24	0.42	1.8	13	0.37	1.7	0.935
EVOO Quant	5	-0.37	1.6	10	-0.55	2.3	0.877	36	0.01	1.8	28	-0.23	1.7	0.595
Vegetables	21	-0.04	1.9	27	-0.6	1.7	0.277	20	-0.19	1.6	11	0.24	2.1	0.527
Fruit	7	-0.81	1.9	9	-0.66	1.5	0.862	34	0.13	1.7	29	-0.21	2.0	0.461
Red Meat	22	-0.3	1.5	25	-0.6	1.8	0.536	19	0.34	2.2	13	0.24	2.0	0.895
Dairy Fat	24	-0.08	1.8	30	-0.4	1.8	0.508	17	-0.1	1.8	8	0.01	2.3	0.899
Wine	1	1.10		1	2.06		-	37	-0.12	1.8	37	-0.38	1.8	0.530
Carb. Beverages	32	-0.2	1.7	33	-0.44	1.8	0.589	9	0.7	1.8	5	0.54	2.1	0.883
Legumes	7	-0.45	1.6	8	-0.20	2.3	0.813	34	0.02	1.8	29	-0.35	1.8	0.413
Fish	11	-0.04	1.7	22	-0.61	1.7	0.375	30	-0.15	1.9	16	0.09	2.0	0.689
Sweets	30	-0.04	1.7	24	-0.33	1.8	0.554	11	-0.18	1.8	14	-0.29	2.0	0.887
Nuts	21	-0.4	1.6	27	-0.45	1.8	0.920	20	0.70	1.9	11	0.02	2.2	0.375
White Meat	22	-0.14	1.6	33	-0.34	1.8	0.673	19	0.28	2.7	5	-0.18	2.6	0.738
Sofrito	11	-0.75	1.6	17	-0.22	2.3	0.503	30	0.42	1.7	21	-0.39	1.5	0.091

Participants were distributed into groups of adherers ('yes' response on checklist) or non-adherers ('no' response on checklist) based on responses to each PREDIMED checklist criteria at end-intervention. Negative values represent an anti-inflammatory DII score and positive values represent a pro-inflammatory DII score.
Abbreviations: DII, Dietary inflammatory index; SD, standard deviation; PREDIMED, Prevención con Dieta Mediterránea; EVOO, extra-virgin olive oil. P-value compares the mean score within groups at each timepoint (i.e., was the score significantly different in adheres from baseline to 12-weeks); independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. * indicates significance ($p < 0.05$).

5.4.5 DII and inflammatory markers and clinical characteristics

Change in serum inflammatory markers and clinical characteristics from baseline to 12-weeks of individuals classified into groups of pro-inflammatory (positive) or anti-inflammatory (negative) change in DII score are presented in **Table 5.6**.

A significant reduction in BMI was observed in the group with a more pro-inflammatory change in DII than the anti-inflammatory change group (-1.17 ± 3.90kg/m^2 vs 0.35 ± 1.84kg/m^2, p =0.048, respectively), likely due to the reduction in total energy (kJ) and carbohydrate intake observed for this group. However, the anti-inflammatory group experienced a significantly greater reduction in visceral fat than the pro-inflammatory group (-2.89 ± 2.18L vs -1.28 ± 2.09L, p =0.039, respectively). This reduction was noted in the absence of any significant weight loss in the anti-inflammatory group. There was a marked improvement, albeit non-significant (p=0.53), in adiponectin levels in the pro-inflammatory change group only.

Table 5.6. Baseline to 12-week change in inflammatory markers, liver outcomes, biochemistry and clinical characteristics across change in DII groups

	Change in DII						
	Anti-inflammatory (-4.4 to 0.0)			**Pro-inflammatory** (0.0 to 6.0)			
Change Variable	n	mean	SD	n	mean	SD	**p**[a]
DII	21	-1.9	1.3	17	1.8	1.7	0.001*
hs-CRP (mg/L)	17	-0.1	1.5	14	-0.1	1.5	0.83
TNF-α (pg/mL)	21	-0.9	5.6	16	-0.5	1.0	0.73
IL-6 (pg/mL)	21	1.3	3.6	16	-0.2	3.3	0.21
Adiponectin (µg/mL)	21	0.3	11.6	16	4.8	11.0	0.53
Leptin (pg/mL)	21	-0.1	4.2	16	-0.7	3.4	0.65
Resistin (pg/mL)	21	-0.9	3.7	16	1.6	8.1	0.47
IHL (%)	16	0.1	4.5	14	-1.3	3.0	0.82
LSM (kPa)	20	-1.1	6.7	16	-1.5	2.9	0.52
Glucose (mmol/L)	21	0.1	1.7	17	-0.4	0.9	0.54
Insulin (mIU/L)	21	-1.0	6.9	17	-3.6	5.8	0.22
HOMA-IR	21	-0.3	2.2	17	-1.4	2.7	0.32
ALT (U/L)	21	-1.7	38.8	17	-8.4	32.8	0.89
AST (U/L)	21	-1.4	20.1	17	-0.9	26.9	0.98
GGT (U/L)	21	-8.2	67.9	17	-24.8	84.6	0.45
ALP (U/L)	21	6.3	16.8	17	-4.5	24.8	0.32
Cholesterol (mmol/L)	21	-0.1	0.7	17	-0.1	0.8	0.73
HDL (mmol/L)	21	0.0	0.1	17	0.0	0.3	0.81
LDL (mmol/L)	21	-0.1	0.6	17	-0.1	0.4	0.91

Triglycerides (mmol/L)	21	0.0	0.6	17	-0.2	0.5	0.14
Weight (kg)	21	-0.5	8.4	17	-1.1	10.7	0.56
BMI (kg/m^2)	21	0.4	1.8	17	-1.2	3.9	0.048*
WC (cm)	21	-0.9	7.0	17	-1.7	10.9	0.79
Fat Mass (%)	21	0.8	4.8	17	-2.3	8.4	0.84
Visceral Fat (L)	20	-2.9	2.2	14	-1.3	2.1	0.039*
BP Systolic (mmHg)	20	-5.5	13.7	16	-3.5	16.4	0.7
BP Diastolic (mmHg)	20	-1.9	8.0	15	-1.6	14.5	0.95

Participants were split into groups of 'pro-inflammatory' (above 0.00) or 'anti-inflammatory' (below 0.00) change in DII over 12-weeks. Negative values represent an anti-inflammatory change in DII score and positive values represent a pro-inflammatory change in DII score. Abbreviations: DII, Dietary inflammatory index; SD, standard deviation; BMI, body mass index; WC, waist circumference; BP, blood pressure; HOMA-IR, homeostatic model assessment-insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IHL, intrahepatic lipid; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6. P[a] p-value for comparing mean scores across groups of DII change; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. * indicates significance ($p < 0.05$).

A moderate, positive and significant ($p < 0.05$) correlation was observed between DII score at end of intervention and total cholesterol, low-density lipoprotein (LDL) cholesterol and serum leptin (**Table 5.7**). There were no significant associations between DII and any other clinical or inflammatory markers at end intervention, as determined by correlational analysis.

Table 5.7. Partial correlations between DII score at the end of intervention with inflammatory markers, liver outcomes, biochemistry and clinical characteristics at the end of intervention, values adjusted for change in energy intake (kJ)

Variable	**Correlation Coefficient r (p)**
DII	-
Weight (kg)	-0.24 (0.148)
BMI (kg/m^2)	-0.15 (0.372)
WC (cm)	-0.26 (0.123)
Fat Mass (%)	0.11 (0.528)
Visceral Fat (L)	-0.26 (0.181)
BP Systolic (mmHg)	0.09 (0.578)
BP Diastolic (mmHg)	0.09 (0.614)
Glucose (mmol/L)	-0.08 (0.624)
Insulin (mIU/L)	0.14 (0.402)
HOMA-IR	0.04 (0.799)
ALT (U/L)	0.35 (0.032)
AST (U/L)	0.29 (0.079)
GGT (U/L)	0.33 (0.044)
ALP (U/L)	0.12 (0.481)
Cholesterol (mmol/L)	0.36 (0.027*)
HDL (mmol/L)	0.14 (0.401)

LDL (mmol/L)	0.33 (0.043*)
Triglycerides (mmol/L)	0.19 (0.250)
IHL (%)	0.20 (0.294)
LSM (kPa)	0.23 (0.187)
hs-CRP (mg/L)	0.24 (0.190)
TNF-α (pg/mL)	-0.23 (0.178)
IL-6 (pg/mL)	0.16 (0.347)
Adiponectin (μg/mL)	0.11 (0.526)
Leptin (pg/mL)	0.39 (0.019*)
Resistin (pg/mL)	0.12 (0.492)

Partial correlations between change in dietary intake variable and change in DII score, controlling for baseline variable value. * Correlation is significant at the 0.05 level (2-tailed). R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations.

No regression analysis was performed for this data due to the lack of strong, significant correlations observed between DII score with inflammatory markers, liver outcomes, biochemistry and clinical characteristics. The strength of associations determined by the correlational analysis provided no premise to investigate the outcomes or to investigate the degree to which independent variables influenced dependent variables.

5.5 Discussion

The present study examines the impact of improved diet quality on DII scores in a pooled cohort of participants with NAFLD who underwent a 12-week MedDiet or low-fat diet intervention. There was no significant change in DII score observed in this cohort from baseline to 12-weeks, therefore the primary hypothesis of this study was not supported. At 12-weeks, DII score was inversely and significantly correlated with PREDIMED score, a surrogate measure of diet quality reflecting adherence to the MedDiet. Moreover, in this group a reduction in DII score was observed in participants who had a moderate to high level of adherence to a MedDiet at 12-weeks. Of note, there was no significant association observed between adhering to PREDIMED targets for dietary components (e.g. consumption of fruit, legumes and nuts) and DII.[365, 599] Additionally, this study found no significant change in circulating inflammatory markers in participants who decreased or increased their DII score after a 12-week diet intervention.

Adherence to the MedDiet pattern, as determined by PREDIMED scores, was inversely associated with DII score following dietary intervention in this group. This finding was positive in that as adherence to the MedDiet increased, this was associated with a decrease in DII score indicative of a more anti-inflammatory dietary pattern, which corroborates existing literature that the MedDiet is anti-inflammatory in nature.[312, 365, 599] Similarly, Mayr et al. (2018) found that dietary adherence, calculated using PREDIMED (termed MEDAS) score, was moderately and negatively correlated with DII at baseline ($r = -0.44$, $p = 0.001$) and strongly and negatively correlated with DII at 6-months

(r =-0.54, p <0.001). To the best of our knowledge, these dietary intervention studies were the first prospective RCTs to study the impact of the MedDiet on DII score in participants diagnosed with chronic conditions. Observational studies have tested associations between markers of disease and DII score, however limited data is available testing the effects of a healthy dietary intervention on the DII. The present study adds to the paucity of literature available for the effect of changes in diet quality on the DII in well-designed diet intervention studies with good measures of adherence and defined anti-inflammatory diet plans. Although dietary adherence and DII score had a significant, moderate and inverse correlation in the present analysis, additional participant numbers would have allowed for increased statistical power and more defined relationships.

The present study identified no significant change in DII score (-0.26 ± 2.38) in the pooled cohort of patients with NAFLD who underwent a 12-week healthy dietary intervention. Conversely, Mayr et al. (2018) found that a pooled cohort analysis of participants with CHD who completed a MedDiet or low-fat diet intervention experienced a mean change in DII score of -0.53 ± 2.65 after 6-months.[599] Although the change observed in the present study was proportionally smaller than that which was observed in the study of patients with CHD, it is important to acknowledge distinct differences in the two studies. Mayr et al. (2018) recruited a larger study sample of 65 participants and had a greater length of intervention which was double in comparison. Their participants were also of a more severe disease phenotype, as they had previously suffered a cardiac event and were now focussing on secondary prevention of CHD, which would perhaps increase their scope for change. The modest decrease in DII score of -0.32 ± 1.86 in the present group after dietary intervention resembles the DII score of the prescribed LFD meal plan (-0.33), more so than the prescribed MedDiet (-4.55), which was recommended in the study. As observed in **Chapter 4** of this thesis, this is reflective of changes in dietary intake that may have improved diet quality however not to the extent of changes that may be observed with optimal adherence to a MedDiet as prescribed or advised. Unlike the present study which assessed a subset (28) of the 45 DII nutrient intake parameters, Mayr et al. (2018) used all 45 parameters in their DII analysis.[365] The nutrient parameters which were excluded in the present analysis (mostly herbs, spices, condiments) had anti-inflammatory effect scores, and therefore the anti-inflammatory potential of diets consumed by participants in this trial may have been underestimated. However, previous research has identified that all 45 DII parameters are not required to calculate the DII score of one's diet and studies that have not included the full set of intake parameters in their analysis still produce valid results.[339, 340, 585, 586, 591]

Dietary intake analysis in the present study showed that participants with greater reductions in DII score had significantly increased their intake of protein, PUFAs, omega-3, linoleic acid, fibre, vitamin C and E, beta-carotene, magnesium, serves of fruit, vegetables and oil equivalents. It should be noted that nutrient parameters (protein, PUFAs, omega-3, linoleic acid, fibre, vitamin C and E, beta-carotene) were all included in calculation of the DII score, hence collinearity must be

considered. These nutrient parameters are considered to have anti-inflammatory effects, a theory supported by the findings of the present study. These findings are supported by another paper published by the AUSMED group which reported similar results, whereby increased intake of nutrients omega-3, fibre, vitamin C and E, and food groups of fruit, vegetables and olive oil were characteristic of participants who experienced a larger anti-inflammatory DII change.[599] Another study associated observed the highest reduction in DII score to occur after 2-months of a vegan dietary intervention, when participants significantly increased intake of fibre and potassium and decreased calcium (likely reduction in dairy products), total and saturated fat consumption.[585] Similar results were also observed in previously mentioned cross-sectional study of MetS which reported a higher DII intake was associated with a lower intake of PUFA, omega-3, fibre, vitamin C and E, and beta-carotene.[589] Additional cross-sectional analyses have also associated lower DII scores with a high consumption of fruit and vegetables.[583, 589, 600] The anti-inflammatory DII scores associated with these diet parameters are consistent with modulating effects of chronic inflammation.[588]

Studies have supported the theory that a reduction in DII score occurs with an increase in the consumption of foods containing anti-inflammatory properties; however, there is limited research to show that this change is reflected in serum inflammatory markers. The results of the present study found no significant change in inflammatory markers of participants who decreased or increased their DII score after a 12-week dietary intervention. To date, there have been no studies examining DII and underlying inflammation in NAFLD. Elevated levels of pro-inflammatory marker CRP (>3 mg/L) have been positively and significantly associated with DII score in healthy individuals [339, 340, 601] and participants with the MetS.[602] In patients with coronary heart disease a higher DII score was associated with increased odds of mean CRP above 3mg/L (at baseline (OR = 1.10, 95% CI 0.87, 1.38) and at 6-months (OR = 1.06, 95% CI 0.81, 1.39), although these results were not statistically significant.[599] Some studies of healthy individuals have demonstrated no association between CRP and DII score both cross-sectionally [603] and after longitudinal follow-up.[342] The same cohort of individuals with the MetS (mentioned above) showed no associations between DII score and circulating level of TNF-α, IL-6 or adiponectin.[602] The markers did not differ significantly between quartiles of DII.[602] Inflammatory marker TNF-α has not been commonly investigated in DII research, even though it is one of the six inflammatory markers used to calculate the overall inflammatory score for each food and nutrient intake parameter included in the DII. The present study did not find any difference in TNF-α between participants with an increase or decrease in DII score. Unlike TNF-α, increased levels of serum IL-6 have been associated a higher DII score in various cohorts of healthy adults.[340, 603, 604] In patients with CHD, AUSMED researchers reported no difference in IL-6 levels between patients in pro- or anti-inflammatory DII groups at baseline.[599] However, after 6-months of diet intervention a reduction in DII score was correlated with a decrease in IL-6.[599] The association found between DII and IL-6, but not CRP, may be related to the mechanism of action of the inflammatory marker. IL-6 is a pleiotropic cytokine, released from

activated cells at the vascular endothelium, playing important role in the causation of atherosclerosis and CVD and stimulation hepatic CRP synthesis.[599, 603]

In this study, DII score was positively associated with serum leptin concentration, cholesterol and LDL-c after adjustment for change in energy intake. Leptin is a pro-inflammatory adipokine, produced by adipose tissue in response to increases in adiposity.[605] Leptin contains pro-inflammatory properties suggested to be similar to those of immune cell-derived cytokines such as tumor necrosis factor alpha (TNF-) and interleukin 6 (IL-6).[606] There is one other study that has reported a positive association between leptin and DII score after adjustment for age, sex, BMI, energy intake, and physical activity.[607] The cross-sectional study of healthy Indonesian adult's study found that plasma leptin concentration increased significantly across tertiles of DII score.[607] Another cross-sectional study in U.S. police officers investigated the relationship between DII score and leptin, finding no significant association.[592] Dyslipidaemia is often an early, identifiable risk factor in patients who develop NAFLD, typically characterised by increased serum triglycerides and low high-density lipoprotein (HDL) cholesterol.[18] Although triglycerides and HDL-c were not associated with DII in this cohort, cholesterol and LDL-c were correlated with DII score indicating the disturbance of lipid profile as DII score increases. Cholesterol is considered a pro-inflammatory nutrient according to its inflammatory effect score on the DII index[338] and whilst no known studies have reported a positive correlation of total cholesterol or LDL-c with DII, studies have reported positive association between DII score and triglyceride,[589] and inverse association between DII score and HDL-c.[608] Previously, dyslipidaemia was used as an early biomarker of NAFLD and cardiometabolic disorders, however increasing evidence indicates that inflammation should be considered a key risk-factor and biomarker for development of these diseases.[607] Collective indictors of disease, such as leptin, triglyceride and cholesterol profile, could better indicate development of oxidative stress and inflammation in obesity prior to the development of NAFLD and NASH, and resultant increased risk for CVD.[609]

Unlike these pro-inflammatory cytokines, adiponectin is an anti-inflammatory adipokine released primarily by adipose tissue, typically low in patients with cardiometabolic risk factors and disease and higher in healthy individuals. An increase in adiponectin concentration is typically observed in response to an improvement in diet quality and reduction in excess adiposity.[610] Results of this study indicate that participants whose diets became more anti-inflammatory, according to DII, experienced a significant reduction in visceral fat (VF) − unaccompanied by weight loss or significant change in adiponectin. Excess visceral adipose is mechanistically responsible for the secretion of biologically active molecules that create a pro-inflammatory environment. Visceral fat surrounds vital organs and is considered the most detrimental to health, due to its up-regulation of the inflammatory process which aids pathogenesis of metabolic diseases T2DM, the MetS, NAFLD and increases risk of CVD.[611] The present study highlights the link between anti-inflammatory potential of diet and reduction in VF. Participants who lowered DII score over the intervention

period were adhering to components of a high-healthy fat MedDiet, such as higher intakes of MUFA, omega-3 PUFA and dietary fibre and lower intake of SFA. Unsaturated fats are associated with higher post-prandial lipid oxidation and thermic effect.[612] Moreover, certain biological compounds of unsaturated fats, such as oleic acid (the predominant MUFA in olive oil), are favourably oxidized compared to others, such as SFAs, which are favourably stored.[611] A possible explanation for the lack of improvement in adiponectin may be that dietary changes made while adhering to the *ad libitum* diets were not substantial enough to evoke change in inflammatory markers. Mayr et al. (2018) reported that an *ad libitum* MedDiet administered to patients with CHD had no significant effect on adiponectin or VF in comparison to a low-fat diet, despite significantly improved MedDiet adherence scores and reduced subcutaneous adipose tissue in the MedDiet group.[613] The lack of significant effect of diet on adiponectin levels may have been attributable to the lack of improvement in VF, as VF represents more metabolically dysfunctional tissue than subcutaneous adipose tissue. Authors also estimated that in the absence of weight loss, twice the sample size would be required to demonstrate significant improvement in concentration of adiponectin.[613] The present and mentioned studies were underpowered to detect a change in inflammation, though trends were in the right direction.

In the present study, circulating adiponectin seemed to increase (i.e., improve) in response to a restriction in total energy and carbohydrate intake in the pro-inflammatory DII group, who also experienced a reduction in BMI. Adiponectin improved in participants who experienced a more pro-inflammatory DII change from 0- to 12-weeks, albeit non-significantly. Although the difference in adiponectin did not differ significantly between pro- and anti-inflammatory DII groups, the magnitude of change was notably different between groups of this underpowered sample. Two studies that investigated the relation between adiponectin and DII score, reported no significant differences in adiponectin levels across quartiles of DII in patients with the MetS[602] and CHD.[599] An inverse association was found between DII score and levels of adiponectin in a study of healthy adults,[603] although this study was cross-sectional and did not assess diet or dietary change. The finding that total energy and carbohydrate restriction seem to exacerbate inflammation, as determined by DII, but may induce weight loss driven improvements in adiponectin is somewhat justified in previous literature. In the absence of weight loss, the MedDiet has been found to significantly reduce inflammation (composite score of CRP, IL-6 and tumour necrosis factor-a),[484] but not levels of adiponectin.[557] The DIRECT study implemented a 6-month MedDiet intervention + weight loss phase followed by an 18-month weight maintenance phase, and found that adiponectin continued to significantly increase in for the duration of the trial.[614] A study of patients with the MetS found that a MedDiet which achieved 10% weight loss significantly improved circulating adiponectin, whereas a MedDiet without weight loss did not affect adiponectin.[557] Literature suggests that the initial significant improvement in adiponectin may be dependent on concomitant weight loss,[613] which helps to explain the lack of improvement in adiponectin in the 'anti-inflammatory DII-change group' of this study who did not experience a reduction in body weight

or BMI. Based on these findings, sustained effects of weight loss (or weight maintenance) and associated effects on adipokine and cytokine markers warrant further investigation. The risk of regaining weight is a major concern in metabolically unhealthy patients, therefore results beyond the initial weight-loss period need to be explored. Ideal intervention may require adherence to an anti-inflammatory diet and physical activity to induce initial weight loss, followed by sustained diet with or without subsequent weight change.

An interesting finding of this study was that a restriction in total energy (kJ) and carbohydrate intake induced weight-loss driven improvements to HOMA-IR and serum adiponectin (as seen in Chapter 4 of this thesis), however these changes were not categorised as anti-inflammatory, in fact they were identified as pro-inflammatory according to DII classifications. More specifically, a reduction in BMI, percentage fat mass and HOMA-IR, and an increase in adiponectin was seen in participants whose DII score increased. These participants also significantly reduced total energy and carbohydrate intake and non-significantly reduced SFAs, a feature of calorie/fat-restricted diets such as the LFD, that is typically administered to patients with NAFLD as standard therapy in a clinical setting. Although these changes induce weight-loss, they do not necessarily contribute to improvements in DII or inflammatory markers. Unlike this, changes to VF may have been induced through adherence to key principles of a MedDiet which as known to attenuate abdominal adiposity by altering adipose tissue composition.[610] A similar finding was presented in the aforementioned ORISCAV-LUX study population, interestingly, participants with higher DII score had significantly lower BMI, waist circumference and systolic blood pressure levels.[590] Authors also state that in their population DII was unrelated to cardiometabolic biomarkers including diastolic blood pressure, lipids, glucose, insulin, HOMA-IR and CRP.[590] These results were inconsistent with findings from the SEASONS validation study, which reported BMI increases with increments in DII score.[339] The ORISCAV-LUX study population had a generally anti-inflammatory mean DII score,[590] indicating that they were perhaps relatively 'health conscious' much the same as the present study group. Based on previous and present study findings, the effect of the DII on body weight remains inconclusive.

The prospective dietary intervention that was administered in patients diagnosed with NAFLD was a major strength of this study and adds to the limited data available for DII score, and inflammatory and liver outcomes. This study included comprehensive measures of novel inflammatory markers and body composition that are not routinely measured in large scale studies and have not been measured in many diet intervention studies investigating the DII, and definitively not in any studies of DII and NAFLD. In particular, studies that measure inflammatory markers are highly valuable and results applicable when discussing the use of the DII score. The measurement of inflammatory potential of diet can only be confirmed by investigating changes in circulating inflammation due to dietary intake, therefore additional studies are required in this area. The present study utilised 3-day food diaries for assessment of dietary intake and calculation of DII score based on food and nutrient

intake parameters. The food diaries had also been checked by an accredited practicing dietitian whilst the participant was present, increasing the accuracy of diet data. Most studies investigating DII use diet data from food frequency questionnaires (FFQs) which are less accurate and are inclined to error. Specific quantities of many food and nutrient parameters are difficult to measure via FFQs and condiments, herbs and spices are not included. Another strength of this diet intervention was the small number of non-completers (n=4) in the study. The diet interventions were administered in a real-world setting with an *ad-libitum* approach, making the diets and the results transferrable to many population groups and settings.

Whilst a strength of this study included dietary intake assessment using 3-day food diaries, this also presented a limitation in that all 45 DII parameters were not included in the final measurement and calculation of the DII score. Only 28 of the 45 food and nutrient parameters were included. The anti-inflammatory potential of changes to DII score as assessed by dietary intake may have been limited by excluded parameters in this study. Aside from this limitation, the small sample size of this cohort also limited findings to reach statistical significance due to the lack of statistical power making it difficult to compare groups and see any definitive changes. Trends were observed in the anti- versus pro-inflammatory groups, as well as in adherers to MedDiet versus non-adherers, and moderate, significant inverse associations were observed. There is scope to increase strength of these findings and associations in a larger patient sample. The DII score of the MEDINA cohort at baseline was relatively "neutral", participants were not consuming a poor diet as was seen in Chapter 1 and 2 of this doctoral thesis through dietary intake analysis. For this reason, it was difficult to measure the true effect of changes in diet quality and anti-inflammatory changes that may have been more pronounced in participants with poorer diets and progressed disease. Although some changes were observed, there was less scope for change in the present sample. While the pooled-analysis of the study cohort increased statistical power and precision of estimate, it is also a limitation of this study as it eliminates the 'control' group from the study design. Further research is required in larger cohorts of individuals undergoing diet intervention which are powered to detect change in diet quality and DII score, and their subsequent effects serum inflammatory markers. The effect of an anti-inflammatory diet on reducing visceral fat which may mediate circulating cytokines and adipokines requires further explanation. There is limited capacity to see a change in inflammation in healthy populations, therefore participants with diseases or disorders with underlying chronic inflammation and/or metabolic disruption should be targeted.

Overall, this study sample did not experience a significant change in DII score following dietary intervention. Adherence to the MedDiet was inversely associated with DII, as were intakes of anti-inflammatory nutrient and food parameters; protein, PUFAs, omega-3, linoleic acid, fibre, vitamin C, vitamin E, beta-carotene, magnesium, serves of fruits, vegetables and oil equivalents. Whilst DII score was not associated with changes in serum inflammatory or biochemical markers, weight loss was greater in participants with increasing DII, but visceral fat reduction was greater in those who

reduced DII − indicating that changes in dietary intake which may lower inflammation (based on DII) could be independent of weight loss and dependent on visceral fat reduction. The relationship between dietary intake, inflammation and body composition is important an contributor to the pathophysiological mechanisms of disease development and requires further investigation in larger cohorts of adults with NAFLD.

5.6 Linking chapter 5 and 6

Thus far, this thesis has examined the baseline inflammatory and clinical profile of patients with NAFLD (Chapter 1), the effects of the MedDiet and LFD intervention on inflammatory and clinical outcomes in all patients with NAFLD (Chapter 2) and the effects of dietary change (either intervention) on the novel, theoretical measurement tool the Dietary Inflammatory Index (DII)(Chapter 3). Considering that low-grade inflammation and the subsequent development of NAFLD is mainly attributed to dietary, environmental, and **genetic** factors, Chapter 6 will investigate the four of the underlying single nucleotide polymorphisms (SNPs) that may affect the synthesis and release of inflammatory markers in response to dietary intake.

The limited number of participants in this sample and comprehensive data collection will allow for an in-depth analysis of the responsiveness to diet from an inflammatory standpoint, as well as the effects on other clinical markers of disease, anthropometry, and body composition. Such investigations will produce novel findings for the field, contribute to the theory underpinning personalised nutrition, and inform more specific effects on the genetic variation which may affect circulating inflammatory markers.

6 Single Nucleotide Polymorphisms in Inflammatory Genes may modify disease-risk and interact with nutrients to modulate serum cytokine levels in patients with NAFLD

6.1 Abstract

Introduction: The pathogenesis of non-alcoholic fatty liver disease (NAFLD) is complex, with chronic inflammation a key metabolic perturbation in its development and progression. Diet, environmental factors and genetic susceptibility contribute to inflammation and disease progression. Single nucleotide polymorphisms (SNPs) coding for inflammatory genes may mediate abnormal cytokine and adipokine levels. Gene-nutrient interactions may be important in modulating inflammation that contributes to the development and progression of NAFLD. **Aim:** to examine the prevalence of SNPs CRP +1846C/T (rs1205), IL-6 -174G/C (rs1800795), TNF-α -308A/G (rs1800629) and adiponectin +276G/T (rs1501299) in NAFLD, and assess the likelihood of developing T2DM and the MetS within gene variants, modulating effects of gene-diet interactions and associations with inflammatory markers after a dietary intervention that improves diet quality.

Methods: Forty-two participants with ultrasound or biopsy proven NAFLD were enrolled in a 12-week, multi-centre, randomised controlled trial and buccal swabs were taken at baseline. Polymorphisms were genotyped by Fitgenes LTD. At baseline and 12-weeks, anthropometry, body composition, biochemistry, liver outcomes and inflammatory markers were measured. Dietary intake was assessed using 3-day food diaries at each timepoint. Diet quality was measured using the 14-item validated PREDIMED checklist. Diagnoses of T2DM and the MetS was completed using patient medical histories. All participants who underwent dietary intervention were combined for this analysis regardless of diet group allocation.

Results: For CRP +1846, 91% of participants were risk allele carriers and for adiponectin +276, 90% were risk allele carriers. For SNP IL-6 -174, the majority did not carry the risk allele, however 57% carried the high producer (GG) phenotype. Gene variants IL-6 -174 and adiponectin +276 were associated with risk of developing the MetS and T2DM, respectively, regardless of diet group allocation. Presence of SNPs TNF-α -308 and CRP +184 predicted levels of their respective serum inflammatory markers following dietary intervention. The risk genotypes of the TNF-α -308 polymorphism and MUFA (%E) intake above the median were associated with reductions in circulating TNF-α. **Conclusion:** Findings of this study add to the limited literature available for the prevalence of SNPs CRP +1846, IL-6 -174, TNF-α -308 and adiponectin +276 in a NAFLD cohort. IL-6 -174 and adiponectin +276 SNPs may predict disease, whereas TNF-α -308 and CRP +184 were indicators for a change in serum inflammatory markers. Findings for the prevalence and function of polymorphism IL-6 -174 were consistent with previous literature. Additional research is warranted for the gene-nutrient interactions that occur within the TNF-α -308 polymorphism as results of this study were significant.

6.2 Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease world-wide, affecting approximately 30% of the total adult population.[571] Rates of NAFLD are rapidly increasing in parallel with rates of diet-related diseases including obesity, insulin resistance (IR) and type 2 diabetes mellitus (T2DM).[92] In its simplest form NAFLD is characterised by fat accumulation (>5%) in the liver, whereas complicated NAFLD consists of steatohepatitis, with or without fibrosis and cirrhosis. Ultimately, the primary causes of mortality in patients with advanced liver disease are hepatocellular carcinoma (HCC) or cardiovascular events.[615] Most researchers in this area agree that the initial "hit" of fat accumulation is not the crucial point of liver injury, but rather the "second hit" of oxidative stress, lipid peroxidation and cytokines that induce inflammation, fibrosis and necrosis.[616] Similar to other metabolic disorders which encompass main systemic components and underlying chronic low-grade inflammation, NAFLD is primarily attributable to dietary, environmental and genetic factors.[17] Genetic variants are associated with increased susceptibility to NAFLD and may influence pathogenesis of IR, regulation of lipid metabolism, oxidative stress and inflammation.[73, 330]

Researchers have studied families and found significant evidence for heritability indicating a link to genetic predisposition for NAFLD, the extent of which is still unknown. In 2001, Willner et al.[70] found familial clustering to be common in a population of non-alcoholic steatohepatitis (NASH), with 18% of participants having a first degree relative affected similarly. In another familial study it was found that family members of overweight children with biopsy-proven NAFLD had higher rates of NAFLD than family members of children who did not have NAFLD.[68] Many of the affected family members were unaware that they were suffering from the disease which is not uncommon considering the asymptomatic nature of NAFLD.[68] Understanding the risk of NAFLD as a hereditary disease will allow for early screening, detection and give patients and health practitioners the ability to employ preventative measures at a stage where the disease is reversible. Initial familial, twin and epidemiological studies provided convincing evidence for genetic contribution in NAFLD,[68, 70, 617] and population-based association studies help to further identify genetic factors accounting for variability in pathogenesis and progression of NAFLD.[330] Evidence for the role of genetic variants implicated in NAFLD was strengthened by a few large multicentre case-control studies which demonstrated that insulin signaling,[618] oxidative stress [619, 620] and fibrogenesis[621] are key functional mechanisms in the progression of NAFLD towards NASH fibrosis.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation that occur in the human genome and are commonly used as potential genetic markers for diseases.[622] Population-based candidate gene and allele association studies use SNPs to identify critical differences in DNA sequence which may contribute to phenotypic variation of specific traits.[330, 623] Studies that assess associations between SNPs and trait differences in large populations have greater power to detect effect, and for this reason genome-wise association studies (GWAS) such as the

International Haplotype Map (HapMap) Project were conducted.[624] Prior to the HapMap project, candidate-gene studies had identified SNPs using small patient cohorts suffering from common diseases (diabetes, NAFLD, cancer, heart disease, stroke, depression and asthma) but findings were based on arbitrary selection of SNPs without the capacity to capture all variations within the gene.[330] Also, at this time deoxyribonucleic Acid (DNA) sequencing was not feasible due to cost. The HapMap project allowed researchers to understand the genome and characterise SNPs by patterns of association, including both linkage disequilibrium (correlation between variants on the same chromosome such that alleles are nonrandomly associated) and haplotype (a combination of alleles at multiple linked loci on a chromosome that are transmitted together).[624] GWAS techniques were a significant advancement in the study and identification of novel genes contributing to various important diseases, however due to the non-hypothesis driven nature of GWAS modifiers of disease progression, biological function and pathogenic mechanisms of disease cannot be examined. In order to understand the complexity of phenotypic activity and confirm causality, detailed candidate-gene association studies of common SNPs linked to NAFLD pathogenesis are needed.

Individuals suffering from the same disease often have a substantial proportion of causative alleles in common. Studies have identified that common SNPs in the Patatin-like phosholipase domain-containing 3 (PNPLA3) and Peroxisome proliferator-activated receptor-alpha (PPAR-α) gene are independently associated with the development of hepatic steatosis via influence of triglyceride (TG) accumulation within hepatocytes.[73] However NAFLD, being a multi-factorial and polygenic metabolic disorder, is influenced by several other genetic variations and pathways to disease. Gene variants have also been identified in NAFLD that may regulate inflammation via production and release of cytokines and adipokines. Existing literature has focused on individual SNPs and allelic associations between risk alleles and inflammation; however, this data is limited by small sample sizes, lack of studies confirming similar findings and the inability consistently to link genetic markers with biomarkers. Further to this, little research has identified the effect of gene variants involved in inflammation with response to dietary treatment in NAFLD. The SNPs investigated in this chapter regulate the release of inflammatory markers high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and adiponectin. The serum concentration of these markers has already been established in this cohort of patients with NAFLD, both at baseline and following a dietary intervention whereby diet quality was improved. The gene variants related to these inflammatory markers and chosen for analysis in this chapter are CRP +1846C/T (rs1205), IL-6 -174G/C (rs1800795), TNF-α -308A/G (rs1800629) and adiponectin +276G/T (rs1501299), respectively.

Acute phase protein, CRP, is synthesized by the liver and regulated by the release of cytokines such as IL-6 and TNF-α, secreted in response to T-cells, macrophages and adipocytes.[625, 626] Levels of CRP rise in response to inflammation and it has been identified as an independent risk factor for obesity-related diseases, including NAFLD. Evidence has shown that levels of circulating CRP are

influenced by both clinical and genetic factors, primarily the *CRP* gene variation. Familial studies established that additive genetic factors influence up to 27-40% of the variance in CRP levels.[69, 627, 628] The T minor allele of the rs1205 SNP in the CRP gene has been associated with lower levels of circulating CRP in healthy individuals[629-631] and individuals at-risk of CVD,[632] however studies in NAFLD are lacking. Interestingly, despite the association between the CRP rs1205 polymorphism and circulating CRP levels, evidence indicates that mutant alleles of interleukin-1 (IL-1) and interleukin-6 (IL-6) genes may upregulate CRP production irrespective of SNP variation in the CRP gene.[633-635] Interleukin-6 (IL-6) is a well-studied pleiotropic cytokine and an important mediator of inflammatory and immune responses and is also involved in glucose and lipid metabolism.[636] Circulating levels of IL-6 primarily control the hepatic acute phase response and activates a network of inflammatory signalling pathways; key factors in the chronic 'inflammatory' state of NAFLD.[637] In humans, the rs1800795 SNP, universally referred to as "-174G/C", in the promoter of the IL-6 gene influences transcriptional regulation and circulating plasma levels of interleukin-6 (IL-6).[636] *In vivo*, carriers of the common allele (G) displayed higher levels of IL-6 in studies of both healthy subjects and patients with inflammatory diseases, including systemic lupus erythematosis and rheumatoid arthritis.[638] However, the IL-6 -174 polymorphism is complex and allelic differences have been identified. Two phenotypes for this polymorphism exist: G/G and G/C genotypes are characterised as 'high-producer' phenotypes wherein higher circulating IL-6 is present; and C/C genotype is the 'low'-producer' phenotype where lower concentrations of IL-6 are observed.[151] Studies investigating this SNP in patients with NAFLD or NASH are lacking but considering that NAFLD is the hepatic manifestation of the metabolic syndrome with insulin resistance the driving factor in its development and progression, studies are warranted.

Tumor necrosis factor-alpha (TNF-α) is another cytokine known to play a central role in the cascade of inflammatory processes that contribute to the pathogenesis of a large variety of illnesses with inflammatory and autoimmune underlying features.[639] TNF-α is associated with a variety of biological processes; it can have beneficial effects such as activating macrophages in host defence against invading microbes during infection and it can have deleterious effects via stimulation of cytokine secretion contributing to cytokine unbalance and altered homeostasis which may result in organ-specific or systemic injury.[639] Transcriptional regulation of the TNF gene is known to modulate the magnitude of secretory response of the cytokine. Genetic variations (SNPs) in the promoter and coding regions of the gene may affect transcription and expression, contributing to elevated TNF-α production and pathogenesis of disease.[639-641] One of these SNPs is located at position -308 in the TNF promoter, involving the substitution of guanine (G) for adenine (A) known as TNF1 (-308G) and TNF2 (-308A) alleles.[639, 640] Initially, *in vitro* studies indicated that the less common TNF2 allele was associated with high TNF-α production[642, 643] and that TNF2 homozygous individuals had significantly higher levels of circulating TNF-α than TNF1 homozygotes.[640, 644] The TNF2 allele was also associated with increased susceptibility to a variety of autoimmune and inflammatory-mediated diseases, such as insulin-dependent diabetes mellitus (IDDM) and

inflammatory bowel disease.[640, 641] In studies of patients with NAFLD and NASH some studies have linked the TNF2 allele to the development and severity of disease,[645] while others have reported no such association.[78] Hence, the association between the −308 TNF polymorphism and NAFLD is still controversial.

Various SNPs in the adiponectin gene (ADIPOQ) have been associated with increased risk of obesity, IR, T2DM and cardiovascular disease (CVD),[646-651] and have been shown to increase or decrease circulating levels of adiponectin.[652] Unlike other inflammatory markers, adiponectin released into the bloodstream is evidenced to have anti-atherogenic, anti-diabetic and anti-inflammatory effects.[653] Adiponectin +276G/T (rs1501299) is one of the most commonly studied SNPs at the ADIPOQ locus, though prevalence rates and associations of this SNP are conflicting between populations depending on ethnicity and underlying disease status. Initially, a meta-analysis of eight case-control studies involving 1639 NAFLD patients and 1426 controls indicated that adiponectin +276G/T was associated with NAFLD and might be related to increased susceptibility to NAFLD.[616] However, a recently updated systematic review and meta-analysis which included eleven studies reported that there was no significant association between ADIPOQ +276G/T and risk of NAFLD.[654] These studies, among others, have identified that the adiponectin +276G/T variant may be linked to low circulating adiponectin and overweight and insulin resistance (IR). Although evidence is accumulating, many studies for adiponectin +276 in NAFLD show no effect for the risk (G) allele on markers of metabolic health, liver enzymes or anthropometry. Conversely, in healthy, nondiabetic people of Greek,[655] Spanish,[652] Korean and Japanese[656, 657] ethnicity the G allele may confer risk, while the T allele has demonstrated a protective role for IR. An interesting result of a 12-week weight loss intervention in the abovementioned nondiabetic Koreans found that GG homozygotes experienced significant decreases in HOMA-IR and increases in adiponectin, which were not shown in carriers of the T allele. Additional studies are required to elucidate such effects of genetic variation in SNP +276 following diet or weight loss.

Variations in reported associations of these gene variants and their effect on metabolic and inflammatory markers could be due to environmental factors, including diet. Gene-nutrient interactions can modify the pro- or anti-inflammatory effects of dietary changes in certain polymorphisms. While the genetic basis of susceptibility to NAFLD and disease progression have begun to be elucidated, very little is known about the effect of genetic predisposition on the response to dietary treatment.[73] Understanding gene-nutrient interactions may increase the manner in which we target intervention studies to those subjects who are more susceptible or resistant to treatment and to better predict the therapeutic success of lifestyle interventions.[658, 659]

The aim of this chapter was to investigate the prevalence of SNPs CRP (rs1205), IL-6 (rs1800795), TNF-α (rs1800629) and adiponectin (rs1501299) in patients with NAFLD. We aimed to assess susceptibility to T2DM and the MetS within gene variants, and to explore the modulating effects of

gene-diet interactions and their associations with serum inflammatory markers after a dietary intervention that improved diet quality, in the pooled cohort of MEDINA study participants.

Objectives

a. To evaluate the prevalence of common single nucleotide polymorphisms (SNPs) CRP +1846C/T (rs1205), IL-6 -174G/C (rs1800795), TNF-α -308A/G (rs1800629) and adiponectin +276G/T (rs1501299) in a multi-ethnic NAFLD cohort.

b. To evaluate associations between the gene variants and susceptibility to T2DM and the MetS.

c. To investigate differences in serum inflammatory markers, liver outcome measures and key biochemical and anthropometric markers between genotypes of inflammatory SNPs at baseline and 12-weeks, following a dietary intervention which improved overall diet quality of patients with NAFLD.

d. To determine if the presence of gene variants can predict levels of serum inflammatory markers following a dietary intervention.

e. To determine whether changes in dietary intake differed between genotypes of inflammatory SNPs, and whether nutrient changes and adherence to the Mediterranean diet pattern may modulate serum inflammatory markers in carriers of risk versus non-risk genotypes.

Hypothesis

It was hypothesised that the risk allele of CRP +1846C/T, IL-6 -174G/C, TNF-α -308A/G and adiponectin +276G/T SNPs would be associated with increased risk of T2DM and the MetS, and risk allele carriers will have less favourable inflammatory, liver and metabolic risk marker profiles and anthropometric measures than non-risk allele carriers at baseline.

After 12-weeks of a healthy dietary intervention, it was hypothesised that risk genotypes of inflammatory SNPs may be more responsive to dietary change, evidenced by more pronounced reductions in serum inflammatory markers, blood biomarkers and liver outcomes. We hypothesize that the anti-inflammatory effects of dietary interventions are due to the interactive effects these components have on genetic susceptibility to inflammation inherent in an individual. We therefore investigated whether there are interactions between markers of nutritional status (anthropometrical markers, biochemical markers and dietary components that were previously associated with inflammation), and SNPs on inflammatory marker concentrations.

6.3 Methods

6.3.1 Study Design and Participants

Forty-two participants who were enrolled in the Mediterranean Dietary Intervention for Adults with Non-Alcoholic Fatty Liver Disease (MEDINA) were included in this study. Sites of recruitment, recruitment methods and eligibility criteria have been previously described in **Chapter 2** of this thesis. Briefly, enrolled participants with ultrasound or biopsy proven NAFLD were genotyped using a buccal swab sample (Fitgenes LTD) at their baseline (0-week) appointment. A detailed description of the MEDINA cohort at baseline is provided in Chapter 3.

Participants enrolled in this study were grouped as either Caucasian and European (Australian, British, Irish, North-West European, Southern or Eastern European) or Asian and Arab (South-East Asian, Chinese Asian, Southern or central Asian, or Middle Eastern).

6.3.2 Diagnosis of Type 2 Diabetes Mellitus and the Metabolic Syndrome

Information regarding participant age, sex and co-morbidities were recorded during the pre-baseline screening questionnaires and correspondence. Further information regarding the diagnosis of co-morbidities (including type 2 diabetes mellitus) and pre-baseline results were extracted from recruitment sites' clinical databases or patient medical histories.

The National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATP III) criteria[254] were used to classify participants in the MEDINA study population as having the MetS if they had three or more of the following criteria at baseline:

- Hypertension: defined by blood pressure ≥130/85 mmHg and/or patients were receiving blood pressure lowering drugs
- Fasting plasma glucose (≥6.1 mmol/L) or patients taking glucose lowering drugs
- Hypertriglyceridemia: defined by fasting plasma triglycerides ≥1.69 mmol/L
- Low HDL-cholesterol: defined by fasting HDL-cholesterol <1.04 for males or <1.29 mmol for females
- Central obesity: defined by waist circumference >88cm for females or >102cm for males.

6.3.3 Dietary Intervention and Timepoints

Full details of the prescribed diets and dietary counselling methods, study duration and data collected at each timepoint have been previously described in Chapter 2. Briefly, participants were randomly allocated to the Mediterranean Diet (MedDiet) or Low-Fat Diet (LFD) for 12-weeks. Accredited Practicing Dietitians (APD) delivered both diets via an *ad-libitum* approach. Both intervention groups received an equal amount of face-to-face and phone call consultation time with their allocated APD. Data collected at the baseline (0-week) and end of intervention (12-week) appointments will be used in this study. No physical activity recommendations were provided to

participants and weight loss was not an intended outcome of the study, however it was not discouraged if it was a personal goal of the participant.

The MedDiet was based on a traditional Cretan Mediterranean Diet,[279, 345] and full recommendations are described in George et al. (2018).[1] The approximate macro and micronutrient composition of the MedDiet was 44% fat (>50% monounsaturated), 36% carbohydrate and 17–20% protein, and up to 5% alcohol. Participants were given several written resources to guide them through the intervention period including a model 2-week meal plan which was created as an example for participants to follow. The meal plan contained key components of the MedDiet, including; high consumption of plant-based foods and wholegrains, fruits and vegetables, moderate consumption of legumes, nuts and oily fish, moderate consumption of fermented dairy products and white/game meats, and decreased consumption of red meat and sweetened or processed foods. Extra-virgin olive oil (EVOO) was the main recommendation to be used as culinary fat and the meal plan for the MedDiet in total was equivalent to approximately 9,400kJ of total energy per day. The LFD intervention was based on the Australian Dietary Guidelines and Heart Foundation recommendations,[101, 356] and the Australian Guide to Healthy Eating was used as an education resource for low-fat cooking methods, portion sizes and low-fat options. The macronutrient composition of the LFD was approximately 30% fat, 50% carbohydrate and 20% protein.

For the purpose of this study participants were analysed as one group, regardless of their allocated diet intervention group. The groups were combined in order to add more statistical power to genotype groups because of the small sample size of the MEDINA cohort. Though the number of participants in each genotype group are small, this study is a preliminary and explorative study of the impact of gene-diet interactions on response and non-response to a healthy diet intervention and the effect of diet on inflammation between genotypes. A pooled cohort analysis was conducted in order to assess whether SNPs in inflammatory genes are associated with concentration of circulating inflammatory markers and whether these are modulated by diet and diet quality.

6.3.4 Genotyping

Cheek cells were collected using buccal swabs (Isohelix Swabs SK1S); and DNA was extracted using Wizard Genomic DNA Purification Kit (Promega,USA) catalogue number A1120 according to manufacturer's protocol. Extracted DNA samples were stored at -20^{o}C in micro-centrifuge tubes until further analysis. The extracted DNA was quantified and checked for purity by Nanodrop spectrophotometer. Thereafter the DNA samples were genotyped on a SNP array using quantitative real-time PCR on a Life Technologies QuantStudio 12K Real Time PCR system using the cycle relative threshold (Crt) method. The reactions were carried out based on two assays, each with two primers and a Taqman probe, one specific to the target SNP. The output from this system is then entered into and analyzed by Life Technologies Copy Caller software (v 2.1).

6.3.5 Study Outcomes

6.3.5.1 Inflammatory markers

Fasting blood samples were drawn from participants at 0- and 12-week appointments, and serum was immediately separated, aliquoted and stored at −80 °C until a full-batch was ready for laboratory analysis. Serum high-sensitivity (hs)-CRP was analysed by the Alfred Hospital Pathology Laboratory in Prahan, Melbourne. Analysis of all other cytokine and adipokine markers was performed using milliplex immunoassay kits (Millipore Corp., Billerica, MD, USA). Measurements of serum cytokines (interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), cat num: HSTCMAG-28SK) and metabolic hormones (Adiponectin, Resistin and Leptin, cat num: HMHEMAG-34K) were tested simultaneously, this methodology has been previously described.[357] The assay was performed according to the manufacturer's instructions and all samples were run in duplicate. This analysis was performed by the doctoral candidate (AR) who was trained and supervised by collaborators at Deakin University, Melbourne, Australia.

6.3.5.2 Liver Outcomes

Proton magnetic resonance spectroscopy (^{1}H-MRS) was used to measure and calculate Intrahepatic Lipid (IHL) content of the liver. ^{1}H-MRS is the gold-standard measurement tool used to diagnose NAFLD and quantify and monitor changes in IHL percentage (%). The test was performed by a qualified radiographer at the Baker Heart and Diabetes Institute located at the Alfred Hospital, Melbourne, Australia. Participants also underwent Transient Elastography (FibroScan®) to measure liver stiffness and fibrosis over the study duration. Each Fibroscan was carried out by a Hepatologist at the Alfred Hospital Gastroenterology Clinic in Melbourne Australia. Liver outcomes were assessed at 0- and 12-week timepoints.

6.3.5.3 Biochemistry

A fasting blood sample was collected on the morning of 0- and 12-week appointments by trained phlebotomy staff at the Alfred Hospital Pathology Clinic. The samples were prepared and analysed according to standard protocol in the Alfred Hospital Pathology Laboratory. Full details of pathology protocol and biomarkers analysed are presented in **Chapter 2** (**Section 2.8.3.2** and **Table 2.4**)..

6.3.5.4 Anthropometry, Body Composition and Haemodynamic Markers

Weight (kg) and height (cm) were measured in duplicate by a trained MEDINA researcher (EG/AR) at each appointment using calibrated scales and a wall-mounted stadiometer. Participants were advised to wear light clothing and remove shoes, keys, coins or other contents of their pockets that would add weight. Waist circumference (cm) was measured over minimal or no clothing using a measuring tape was placed between the 12th rib and iliac crest. Fat mass percent and visceral fat (L)

were measured using Bioelectrical Impedance Analysis (BIA). Standard procedures were followed by a trained researcher using the Alfred Hospital Nutrition Department Seca© machine.

6.3.5.5 Dietary Intake

Dietary intake was recorded by participants using food records in the form of 3-day food diaries. Participants were required to present their food diaries at the baseline and 12-week timepoints during which the food records were reviewed by a dietitian and any discrepancies were clarified. The records were required to reflect foods consumed on two weekdays and one weekend day, specifying the type, brand, quantity and cooking methods of food and beverage items quantified in household measures. Participants were asked to classify whether or not each day recorded represented a 'normal' daily intake and if it did not (i.e., was an abnormal day of food consumption) then the data was not used. Data from 3-day food diaries were entered and analysed in FoodWorks9™ using the most up to date *AUSNUT 2013, AusBrands 2015* and *AusFoods 2015* databases. Macronutrient, micronutrient and food group analysis was exported from FoodWorks9™ and further analysis was conducted in SPSS® statistical package version 25 (IBM Corp, Released 2017).

6.3.5.6 Adherence to the Mediterranean Diet

Adherence to a Mediterranean Diet was assessed using a 14-point checklist which was developed and validated by MedDiet researchers in Spain, who were investigating the effects of a MedDiet in cardiovascular disease (PREDIMED Study).[660] The PREDIMED score was used to determine changes in diet quality of participants in the present study. The checklist is comprised of key components (foods and beverages) characteristic of a traditional Mediterranean Diet, each item is worth one point for inclusion or zero for exclusion from diet. Therefore, greater adherence will score higher in points on the 14-point checklist and less adherence will score closer to '0'. An example of this checklist is presented in **Appendix 6.2**. The checklist was completed by each participant in the MedDiet group prior to each face-to-face appointment on the trial and the checklist as reviewed by the consulting dietitian during their dietary consult to ensure participants understood the checklist and to minimise errors or inconsistencies. For the LFD group, MedDiet adherence scores were calculated retrospectively so that comparisons could be made between groups for MedDiet adherence.

6.3.6 Statistical analysis

This study represents an exploratory analysis of the MEDINA study cohort and therefore a sample size calculation was not performed prior to collecting the SNP data and conducting analysis. The full cohort of participants were combined for this analysis regardless of allocated diet group. Data presented is based on 42 participants at baseline and 39 participants at the end of the intervention period. The 12-week timepoint and change analyses (i.e., baseline to 12-weeks) were based on

participants who completed the intervention only. The decision to combine participants from MedDiet and LFD groups was based on: (i) the overall improvement in diet quality observed in chapter 4 of this thesis; (ii) to add statistical power to genotype groups given the small overall sample size.

Statistical analysis of inflammatory markers, biochemical, anthropometric, body composition and haemodynamic variables, dietary intake (3-day food diaries) and adherence to the MedDiet (PREDIMED scores) were performed by the doctoral candidate (AR) using the Statistical Package for the Social Sciences (SPSS), version 25 (IBM Corp, Released 2017) and verified by geneticist, Dr Chee Kai Chan. Normality of variables was assessed using Kolmogorov-Smirnov statistic and normality was indicated by a non-significant result ($p > 0.05$). Based on normality, an independent samples t-test or Mann-Whitney U test were used to compare pre post differences in continuous variables. Results were presented as mean ± standard deviation (SD) for comparative purposes and n (%) was used to present frequency.

The statistical analysis of allelic frequencies of the gene variants was performed using R software for Mac, version 3.3.2. Univariate and multivariate logistic regression, odds ratio (OR) and 95% confidence interval (CI) and Hardy Weinberg Equilibrium for each variable were completed using SNPStats package (version 1.24.0) for R. p-value< 0.05 was considered statistically significant. The association between SNPs and T2DM or the MetS was analysed using the co-dominant, dominant, recessive and overdominant modes of inheritance. Recessive genetic models were used to group genotypes for SNPs CRP +1846 and adiponectin +276, and dominant genetic models were used to group genotypes for SNPs IL-6 -174 and TNF-α -308.[661] 'Risk' and 'non-risk' alleles were determined using published literature and established associations with disease or susceptibility to disease, described in **Table 6.1**.

Categorical variables were expressed as frequency (n) and percentages (%). Chi square tests were used to determine group differences for categorical variables, the level of statistical significance was set at $p<0.05$. For a 2 by 2 table the Continuity Correction value was used. For a 2 by 2 table that violated this assumption, the Fisher's Exact Probability test was used instead. Analysis of inflammatory markers, biochemical, anthropometric, body composition and haemodynamic variables at baseline and end-intervention timepoints and changes in dietary intake were performed with genotypes dichotomised using the aforementioned genetic models. To determine if there were significant differences within genotype groups from baseline to end intervention, paired samples t-tests were used for parametric data and Wilcoxon Signed Rank Tests were used for non-parametric data ($p <0.05$). For each SNP, genotype groups remained separated for the analysis of dietary intake and mean change in macronutrient from baseline to end intervention. To test differences in macronutrient intake at pre-and post-intervention, paired samples t-tests were used for parametric data and Wilcoxon Signed Rank Tests were used for non-parametric data ($p <0.05$). To determine whether the mean change in nutrient intake was significantly different between groups, independent

samples t-tests were used for parametric data and Mann-Whitney U Tests were used for non-parametric data ($p < 0.05$).

Univariate linear regression analyses were used to determine if the presence of an individual SNP predicted serum inflammatory marker levels at 12-weeks. Covariates included in the models were age, sex, BMI and baseline levels of inflammatory marker. Genotype groups remained dichotomised as per genetic models mentioned above and were entered as categorical variables with the reference or non-risk genotype variable coded as 0.00, and risk genotype variable coded as 1.00. For each regression model, non-parametric variables; hs-CRP and IL-6 were included as log-transformed variables in order to conform to normality. Before interpreting the results, a number of assumptions were tested, and checks were performed. Assessment of the normal probability plot of standardised residuals and the scatterplot of standardised residuals against predicted values indicated that the assumptions of normality, linearity and homoscedasticity were met. Potential violations of the assumption of multicollinearity was assessed by collinearity diagnostic values for Tolerance and variance inflation factor (VIF). Standardised beta (β) regression coefficients, correlation (R), squared multiple correlation (R^2), adjusted R^2, significance level (P-value) and 95% confidence interval (CI) were reported for each model.

Two-way between groups analysis of variance (ANOVA) models were used to test for associations between individual SNPs and inflammatory markers. Gene-nutrient interactions were tested for using SNP-nutrient interaction terms in univariate general linear models. For nutrient variables at 12-weeks, the median was determined. New variables were then created, where the cohort was dichotomised, scoring 1 for a value below the median and 2 for a value above the median for each nutrient measure. The cohort was then split based on this new dichotomous variable, and a two-way between groups ANOVA was carried out to assess the interaction between high or low intake of the nutrient variable with changes in individual inflammatory markers from baselines to 12-weeks. Thus, this showed cases where the effect of genotype on a serum inflammatory marker could be altered by nutrient intake.

Gene-diet interactions were also tested for using SNP-MedDiet interaction terms in univariate general linear models, where adherence to the MedDiet was determined using PREDIMED score. The mean score of adherence to the MedDiet at 12-weeks was determined and the cohort was then dichotomised, scoring 1 for low to moderate adherence (4.00-7.65) and 2 for moderate to high adherence (7.66-14.00). A two-way between groups ANOVA was carried out to assess the interaction between high or low adherence to the MedDiet with changes in individual inflammatory markers from baselines to 12-weeks. Thus, this showed cases where the effect of genotype on a serum inflammatory marker could be altered by dietary adherence.

Table 6.1. Candidate SNPs and their descriptions

rs number	Gene	Risk allele	Function
rs1205	CRP	C	Shown to be an important determinant of CRP levels. The minor allele (TT or CT) of rs1205 has been associated with lower CRP concentrations in healthy adolescents,[629] healthy adults,[630, 631] and adults at-risk of CVD.[632]
rs1800795	IL-6	C	Influences transcriptional regulation and circulating plasma levels of IL-6,[636] production depends on genotype: G/G and G/C genotypes result in higher circulating IL-6 and C/C genotype tend to have lower circulating IL-6.[151] C allele has been associated with diabetes (two-fold) [662] and anthropometric markers of the MetS.[663] C allele associated with risk factors for CVD, also risk factors for the MetS and NAFLD.[664] Frequency of alleles differs between ethnic populations whereby the G allele is more prevalent in non-Caucasian populations[186, 665] and the C allele is more prevalent in Caucasian populations.[666]
rs1800629	TNF-α	A	May affect transcription and expression, contributing to elevated TNF-α production.[639-641] TNF- α -308 SNP is one of the most commonly studies TNF gene variants, alleles are named TNF1 (-308G) and TNF2 (-308A) alleles.[639, 640] Increased levels of TNF-α are observed in individuals carrying TNF2 homozygotes compared to TNF1 homozygotes.[640, 644] TNF2 carriers had 23% higher risk of developing obesity and significantly higher insulin levels compared with controls.[667] TNF2 carriers had significantly higher risk of NASH development; OR=1.69 [95% CI: 1.05-2.71], are at increased risk for HCC; OR=3.23 [95% CI: 1.10-9.44] and at higher risk for hepatic fibrosis and more severe liver damage.[668, 669]
rs1501299	Adiponectin	G	Conflicting results reported for this SNP. One meta-analysis reported adiponectin +275G/T was associated with NAFLD and might be related to increased susceptibility to NAFLD.[192] A more recent systematic review and meta-analysis reported that there was no significant association between ADIPOQ +276G/T and risk of NAFLD.[654] GG genotype associated with impaired blood glucose and significantly higher IR than carriers of the T allele.[652, 657] T allele was found to be protective for IR.[656, 670] Different responses of circulating adiponectin and IR in GG homozygotes compared to T allele carriers following mild weight loss in non-diabetic, overweight and obese participants.[657]

Abbreviations: CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, tumor necrosis factor-alpha; CVD, cardiovascular disease; MetS, the Metabolic Syndrome; NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-Alcoholic Steatohepatitis; HCC, hepatocellular cancer; SNP, single nucleotide polymorphism; IR, insulin resistance.

6.4 Results

6.4.1 Genotype and allele frequency of SNPs CRP +1846C/T, IL-6 -174G/C, TNF-α -308A/G and adiponectin +276G/T in a NAFLD cohort

The prevalence of the genotypes for each SNP were 48% (n=20) CC, 43% (n=18) CT and 9% (n=4) TT for CRP +1846; 57% (n=24) GG, 31% (n=13) GC and 12% (n=5) CC for IL-6 -174; 75% (n=32) GG, 21% (n=9) GA and 2% (n=1) AA for TNF-α -308; and 10% (n=4) TT, 33% (n=14) GT and 57% (n=24) GG for adiponectin +276.

C was the major allele for CRP +1846 (C frequency = 0.69), G the major allele at IL-6 -174 (G frequency = 0.73), G the major allele for TNF-α -308 (G frequency = 0.87) and G the major allele at adiponectin +276 (G frequency = 0.74), data presented in **Table 6.2**.

Table 6.2. Allele and genotype count and frequency for CRP +1846, IL-6 -174, TNF-α -308, and adiponectin +276 gene variants

	CRP +1846			**IL-6 -174**			**TNF-α -308**			**Adiponectin +276**		
Allele	C	T		G	C		G	A		G	T	
Count (n)	58	26		61	23		73	11		62	22	
Proportion	0.69	0.31		0.73	0.27		0.87	0.13		0.74	0.26	
Genotype	CC	CT	TT	GG	GC	CC	GG	GA	AA	GG	GT	TT
Count (n)	20	18	4	24	13	5	32	9	1	24	14	4
Proportion	0.48	0.43	0.10	0.57	0.31	0.12	0.76	0.21	0.02	0.57	0.33	0.1

For all further statistical analysis throughout this chapter, the genotypes of minor alleles were combined for all SNPs. The SNPs were combined as follow: TT homozygote and CT heterozygote (carriers of the T allele) as one group and CC homozygote genotype as another group for CRP +1846, CC homozygote and GC heterozygote (carriers of the C allele) as one group and GG homozygote genotype as another group for IL-6 -174, AA homozygote and GA heterozygote (carriers of the A allele) as one group and GG homozygote genotype as another group for TNF-α -308, and TT homozygote and GT heterozygote (carriers of the T allele) as one group and GG homozygote genotype as another group for adiponectin +276.

In the analyses below, an asterisk * represents the risk genotype for each gene variant. Risk genotypes were grouped based on previously established groupings found in published literature;

(i) CC homozygote for CRP +1846, (ii) C allele carriers (GC/CC) for IL-6 -174, (iii) A allele carriers (GA/AA) for TNF-α -308, and (iv) GG homozygote for adiponectin +276.

For IL-6 -174, although GG homozygotes have been identified as high producer phenotypes, the most recent research shows that the production of IL-6 varies between tissue type and the C allele has been most consistently associated with higher levels of IL-6 and risk of diet-related disease, especially for Caucasians.[185, 664, 671-674]0 Therefore, the C (minor) allele was considered the risk allele in this study and the CC/GC genotypes were grouped.

Genotype distributions presented as percent prevalence of genotypes for each SNP are presented in **Figure 6.1.**

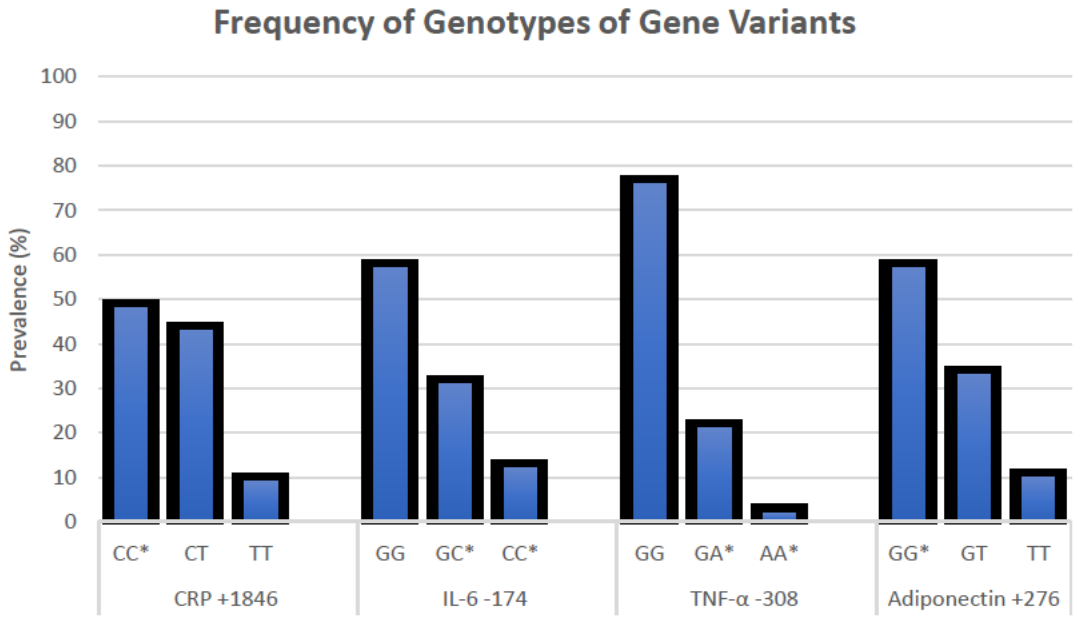


Figure 6.1. Frequency of genotypes of common single nucleotide polymorphisms (SNPs) CRP +1846C/T (rs1205), IL-6 -174G/C (rs1800795), TNF-α -308A/G (rs1800629) and adiponectin +276G/T (rs1501299)

Footnote 1 *indicates risk allele or genotype.

6.4.2 Baseline characteristics of the study population according to genotype of SNPs CRP +1846C/T, IL-6 -174G/C, TNF-α -308A/G and adiponectin +276G/T

Table 6.3 presents the sex distribution, age and distribution of ethnicity between genotype groups of SNP variants; there were no significant differences in sex distribution and age between genotype groups. IL-6 -174GG was significantly more prevalent (p =0.041) in Asian and Arab participants than in Caucasian and European participants. Prevalence of the C allele for IL-6 -174 (GC/CC) was significantly greater (p =0.03) in Caucasian and European participants than in Asian and Arab

participants. TNF-α -308GA/AA was significantly more prevalent (p =0.016) in in Caucasian and European participants than in Asian and Arab participants.

Table 6.3. Baseline characteristics of the NAFLD cohort, split by candidate SNPs and genotypes

	CRP +1846C>T		**IL-6 -174G>C**		**TNF-α -308A>G**		**Adiponectin +276G>T**	
	CC*	CT/TT	GG	GC/CC*	GG	GA/AA*	GG*	TT/GT
n	20	22	24	18	32	10	24	18
Sex								
Male (n)	9	8	10	7	14	3	10	7
Female (n)	11	14	14	11	18	7	14	11
Age (y)	49.3 ± 14	55.1 ± 10	51.6 ± 12	53.3 ± 14	51.4 ± 13	55.5 ± 12	52.1 ± 13	52.1 ± 12
Ethnicity								
Caucasian and European	12 (60%)	9 (41%)	10 (38%)	**11 (69%)** [b]	14 (44%)	**7 (70%)** [b]	13 (54%)	8 (44%)
Asian and Arab	8 (40%)	13 (59%)	**16 (62%)** [b]	5 (31%)	18 (56%)	3 (30%)	11 (46%)	10 (56%)

[a] mean ± SD. *genotypes of minor alleles combined for analysis.
[b] group differences were calculated using the chi-square test on categorical variables (p<0.05).

6.4.3 Associations between gene variants with risk factors of the Metabolic Syndrome

Of the 42 participants with NAFLD, 23 participants (55%) met the NCEP ATP III[402] criteria for the MetS. The HWE test of independence showed that case (MetS) and control (no MetS) groups for all gene variants were independent (p >0.05), therefore they were in equilibrium and it was possible to perform further tests on associations.

A significant association between the IL-6 -174 polymorphism and presence of the MetS was demonstrated under the dominant (GC/CC vs GG: OR = 4.78, 95% CI = 1.20-18.98, p =0.021) and overdominant (GC vs GG/CC: OR = 4.92, 95% CI = 1.02-23.63 p =0.036) inheritance models. The G/C and G/C-C/C genotypes of IL-6 – 174 increased risk of MetS in codominant, dominant and over-dominant inheritance models, as demonstrated in **Table 6.4**.

The CRP +1846, TNF-α -308 and adiponectin +276 polymorphisms were not associated with the MetS in codominant, dominant, recessive or overdominant tested inheritance models in this population (data not shown).

Table 6.4. Association of IL-6 -174 polymorphism with the likelihood of having the Metabolic Syndrome in patients with NAFLD

Mode of inheritance	MetS n (%)	No MetS n (%)	OR (95% CI)	P-value	AIC	BIC
Codominant						
G/G	17 (73.9%)	7 (36.8%)	1.00	0.061	62.4	72.8
G/C	4 (17.4%)	9 (47.4%)	**5.85 (1.18-28.97)**			
C/C	2 (8.7%)	3 (15.8%)	3.16 (0.40-24.74)			
Dominant						
G/G	17 (73.9%)	7 (36.8%)	1.00	0.021*	60.7	69.4
G/C-C/C	6 (26.1%)	12 (63.2%)	**4.78 (1.20-18.98)**			
Recessive						
G/G-G/C	21 (91.3%)	16 (84.2%)	1.00	0.52	65.6	74.3
C/C	2 (8.7%)	3 (15.8%)	1.91 (0.27-13.62)			
Overdominant						
G/G-C/C	19 (82.6%)	10 (52.6%)	1.00	0.036*	61.6	70.3
G/C	4 (17.4%)	9 (47.4%)	**4.92 (1.02-23.63)**			
Log-additive	---	---	2.50 (0.93-6.68)	0.055	62.3	71

The associations between SNPs and the MetS status outcome were evaluated using unadjusted and adjusted ORs (Odds Ratios), 95% CIs and p-values. *Statistically significant; p-value ≤0.05. Abbreviations: MetS, Metabolic Syndrome; AIC, Akaike's Information Criteria; BIC, Bayesian Information Criteria.

6.4.4 Associations between gene variants with Type 2 Diabetes Mellitus

In the NAFLD group, 18 participants (43%) were diagnosed with T2DM. The HWE test of independence showed that case (T2DM) and control (no T2DM) groups for all gene variants were independent (p >0.05), they were in equilibrium and further tests on associations were performed.

A significant association between adiponectin +276 and having T2DM was demonstrated under the codominant (GT vs TT: OR = 0.04, 95% CI = 0.00-0.82, p =0.025), dominant (GT/TT vs GG: OR = 0.11, 95% CI = 0.01-0.85, p =0.016) and over-dominant (GT vs GG/TT: OR = 0.05, 95% CI = 0.00-0.85, p =0.0083) inheritance models. The G/T and G/T-T/T genotypes of adiponectin +276 lowered risk of T2DM in codominant, dominant and over-dominant inheritance models, as demonstrated in **Table 6.5**.

The CRP +1846, IL-6 -174 and TNF-α -308 polymorphisms were not associated with T2DM in codominant, dominant, recessive or overdominant tested inheritance models in this population (data not shown).

Table 6.5. Association of adiponectin +276 polymorphism with type 2 diabetes mellitus in patients with NAFLD

Mode of inheritance	T2DM n (%)	No T2DM n (%)	OR (95% CI)	P-value	AIC	BIC
Codominant						
C/C	7 (38.9%)	17 (70.8%)	1.00	0.025*	43.9	54.3
C/A	9 (50%)	5 (20.8%)	**0.04 (0.00-0.82)**			

A/A	2 (11.1%)	2 (8.3%)	0.41 (0.03-5.95)			
Dominant						
C/C	7 (38.9%)	17 (70.8%)	1.00	0.016*	43.5	52.2
C/A-A/A	11 (61.1%)	7 (29.2%)	**0.11 (0.01-0.85)**			
Recessive						
C/C-C/A	16 (88.9%)	22 (91.7%)	1.00	0.9	49.3	58
A/A	2 (11.1%)	2 (8.3%)	0.85 (0.07-10.54)			
Overdominant						
C/C-A/A	9 (50%)	19 (79.2%)	1.00	0.0083**	42.4	51.1
C/A	9 (50%)	5 (20.8%)	**0.05 (0.00-0.85)**			
Log-additive			0.32 (0.08-1.32)	0.08	46.3	54.9

The associations between SNPs and T2DM status outcome were evaluated using unadjusted and adjusted ORs (Odds Ratios), 95% CIs and p-values. *Statistically significant; p-value ≤0.05, **statistically significant; p-value ≤0.001. Abbreviations: T2DM, Type 2 Diabetes Mellitus; AIC, Akaike's Information Criteria; BIC, Bayesian Information Criteria.

6.4.5 Differences in inflammatory, biochemistry and haemodynamic markers, liver outcomes, and anthropometric and body composition variables across the genotypes at baseline and post 12-week dietary intervention

As shown in **Table 6.6**, T allele carriers (TT and CT) of CRP +1846 had significantly lower levels of circulating serum hs-CRP (p =0.048) than CC* homozygotes.

Following dietary intervention to improve the overall quality of participant's habitual dietary patterns, serum concentrations of hs-CRP (p =0.028) and systolic blood pressure (p =0.047) significantly decreased in CC* homozygotes at SNP CRP +1846, whereas these effects were not observed in carriers of the T allele. There was a noteworthy, albeit non-significant, increase (improvement) in adiponectin concentrations in CC* homozygotes, while the change observed in carriers of the T allele was unremarkable. Measures of visceral fat (VF) decreased significantly in both CC* homozygotes and carriers of the T allele, however these changes were of a higher magnitude in CC* homozygotes (p <0.001) compared to carriers of the T allele (p =0.006).

Table 6.6. Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to CRP +1846C/T genotype

	CRP +1846C/T			
	Carriers of the T allele		*CC homozygotes**	
	Baseline (n = 22)	End Int (n = 20)	Baseline (n = 20)	End Int (n = 19)
Inflammatory Markers				
hs-CRP (mg/L)	2.6 ± 2.49†	2.8 ± 2.55	4.1 ± 2.46	3.1 ± 2.26°
TNF-alpha (pg/mL)	6.1 ± 6.65	4.3 ± 2.31	3.9 ± 1.46	4.4 ± 1.59
IL-6 (pg/mL)	12.4 ± 16.67	15.4 ± 19.63	9.9 ± 19.73	12.1 ± 21.22
Adiponectin (µg/mL)	15.2 ± 11.33	16.8 ± 14.61	15.9 ± 13.42	20.0 ± 20.33
Leptin (ng/mL)	15.7 ± 12.39	14.5 ± 9.71	16.9 ± 12.18	16.1 ± 13.90
Resistin (ng/mL)	37.2 ± 16.11	36.8 ± 16.26	42.3 ± 21.24	42.2 ± 20.32
Liver Outcomes				
IHL (%)	8.7 ± 6.36	7.9 ± 6.93	14.2 ± 11.53	13.1 ± 12.86
LSM (kPa)	9.2 ± 9.81	9.3 ± 8.73	10.9 ± 12.42	9.2 ± 7.91
Biochemistry				
Glucose (mmol/L)	6.4 ± 1.74	6.2 ± 1.18	6.2 ± 2.03	6.4 ± 2.58
Insulin (mIU/L)	18.1 ± 9.98	15.6 ± 9.43	18.7 ± 12.26	16.4 ± 10.80
HOMA-IR	5.5 ± 4.02	4.3 ± 2.76	5.6 ± 5.50	5.3 ± 5.65
ALT (U/L)	55.9 ± 31.54	55.6 ± 37.53	60.6 ± 33.28	54.6 ± 25.27
AST (U/L)	35.8 ± 18.26	39.6 ± 27.21	38.9 ± 19.27	33.6 ± 13.70
GGT (U/L)	112.4 ± 87.13	114.8 ± 97.35	107.6 ± 129.68	83.9 ± 57.45
ALP (U/L)	90.5 ± 25.75	94.7 ± 28.97	94.6 ± 35.71	96.2 ± 26.98
Cholesterol (mmol/L)	4.7 ± 1.35	4.7 ± 1.32	5.2 ± 1.74	5.2 ± 1.62
HDL (mmol/L)	1.3 ± 0.26	1.2 ± 0.31	1.1 ± 0.22	1.1 ± 0.23
LDL (mmol/L)	2.7 ± 1.15	2.7 ± 1.16	3.4 ± 1.40	3.3 ± 1.40
Triglycerides (mmol/L)	1.8 ± 0.84	1.6 ± 0.59	1.8 ± 0.93	1.7 ± 0.86
Anthropometry				
Weight (kg)	87.7 ± 27.94	86.1 ± 22.96	90.1 ± 15.81	88.7 ± 17.39
BMI	31.9 ± 8.07	31.2 ± 6.29	32.5 ± 3.55	31.9 ± 3.74
WC (cm)	106.0 ± 21.05	103.6 ± 15.79	108.3 ± 11.52	106.5 ± 11.52
FM (%)	40.8 ± 7.72	39.6 ± 6.97	39.3 ± 8.08	38.2 ± 8.52
VF (l)	3.6 ± 2.01	1.7 ± 1.43°°	4.2 ± 1.81	1.6 ± 0.50°°°
Blood Pressure				
Systolic (mmHg)	126.6 ± 18.47	121.5 ± 9.58	126.5 ± 13.90	120.1 ± 14.39°
Diastolic (mmHg)	82.0 ± 9.21	82.7 ± 8.20	84.3 ± 7.93	81.2 ± 8.85

All data presented as mean ± standard deviation. ° indicates statistical significance; ° P <0.05, °° P <0.01, °°° P <0.001, difference between timepoints within the genotype group. † represents significant difference in baseline values across groups and end intervention values across groups; P<0.05, ††P<0.01.

For IL-6 –174 (**Table 6.7**), carriers of the C* allele (CC and GC) had significantly higher levels of inflammatory markers adiponectin (p =0.034) and leptin (p =0.024) than GG homozygotes at baseline. Carriers of the C* allele were significantly heavier (kg) (p =0.047) and had a significantly higher BMI (p =0.034) and WC (p =0.045) than GG homozygotes at baseline.

No significant change was observed in serum inflammatory markers according to GG homozygote and C* allele carrier genotypes at SNP IL-6 – 174. Intrahepatic lipid content (%) decreased significantly in carriers of the C* allele (p =0.041) at SNP IL-6 – 174, whereas increased non-significantly in GG homozygotes. Conversely, LSM (kPa) decreased significantly in GG homozygotes (p =0.013) and did not change significantly in carriers of the C* allele. There were significant decreases in VF in both GG homozygotes (p <0.001) and carriers of the C* allele (p =0.013). Although carriers of the C* allele experienced more of a (non-significant) reduction in body weight compared to GG homozygotes (Δ-2.8kg vs Δ-0.0kg, respectively), carriers of the C* allele remained heavier (kg) (p =0.046), and had a higher BMI (p =0.008), WC (p =0.022) and FM (p =0.003) than GG homozygotes after the dietary intervention period. Interestingly, serum concentrations of adiponectin were significantly higher in carriers of the C* allele than GG homozygotes at end of intervention (p =0.005), similarly with baseline levels.

Point-biserial correlations were run to determine the relationship between variables at each timepoint with IL-6 -174 genotypes. At end intervention, there was a moderate, positive correlation observed between BMI and SNP IL-6 -174, which was statistically significant (*rpb = 0.321, n = 39, p = 0.047*). The results of this analysis are presented in **Appendix 11; Supplementary Table 7,** represented as a box plot which displays the distribution of variables between risk and non-risk genotypes.

Table 6.7. Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to IL-6 -174G/C genotype

	IL-6 -174G/C			
	*Carriers of the C allele**		*GG homozygotes*	
	Baseline (n = 18)	End Int (n = 16)	Baseline (n = 24)	End Int (n = 23)
Inflammatory Markers				
hs-CRP (mg/L)	4.1 ± 2.73	3.5 ± 2.85	2.7 ± 2.36	2.6 ± 2.04
TNF-alpha (pg/mL)	6.5 ± 7.31	4.4 ± 2.27	4.0 ± 1.53	4.3 ± 1.78
IL-6 (pg/mL)	7.8 ± 9.66	11.7 ± 15.18	13.5 ± 22.07	15.3 ± 23.30
Adiponectin (µg/mL)	20.4 ± 13.55†	23.7 ± 14.09††	12.2 ± 10.21	14.6 ± 18.86
Leptin (ng/mL)	21.2 ± 13.20†	18.7 ± 12.86	12.8 ± 10.26	12.9 ± 10.65
Resistin (ng/mL)	43.2 ± 15.32	43.2 ± 14.23	37.2 ± 20.77	36.8 ± 20.56
Liver Outcomes				
IHL (%)	12.1 ± 12.09	9.2 ± 10.97°	10.9 ± 7.71	11.2 ± 10.24
LSM (kPa)	9.6 ± 11.97	9.3 ± 7.82	10.3 ± 10.51	9.2 ± 8.67°

Biochemistry				
Glucose (mmol/L)	6.4 ± 1.89	6.1 ± 1.34	6.2 ± 1.87	6.4 ± 2.32
Insulin (mIU/L)	20.3 ± 13.04	17.9 ± 13.11	16.9 ± 9.18	14.6 ± 7.11
HOMA-IR	6.5 ± 5.99	5.3 ± 5.24°	4.9 ± 3.49	4.3 ± 3.74
ALT (U/L)	58.2 ± 31.15	46.9 ± 22.78°	58.1 ± 33.41	60.8 ± 36.09
AST (U/L)	38.3 ± 20.37	33.1 ± 18.78	36.5 ± 17.54	39.1 ± 23.52
GGT (U/L)	112.4 ± 95.22	101.7 ± 88.25	108.3 ± 118.85	98.4 ± 77.42
ALP (U/L)	91.9 ± 28.47	98.5 ± 32.75	92.9 ± 32.66	93.3 ± 24.04
Cholesterol (mmol/L)	4.6 ± 1.21	4.6 ± 1.12	5.3 ± 1.72	5.1 ± 1.68
HDL (mmol/L)	1.3 ± 0.28	1.3 ± 0.30	1.1 ± 0.21	1.1 ± 0.24
LDL (mmol/L)	2.7 ± 1.09	2.7 ± 1.09	3.3 ± 1.44	3.2 ± 1.41
Triglycerides (mmol/L)	1.5 ± 0.63	1.5 ± 0.59	1.9 ± 1.00	1.8 ± 0.79
Anthropometry				
Weight (kg)	97.7 ± 25.82†	94.9 ± 22.06†	82.2 ± 17.94	82.2 ± 17.47
BMI	34.7 ± 7.41†	34.3 ± 5.66†	30.3 ± 4.55	29.6 ± 3.83
WC (cm)	114.1 ± 20.58†	111.3 ± 15.55†	101.9 ± 11.72	100.6 ± 10.65
FM (%)	42.5 ± 9.10	43.1 ± 7.02††	38.3 ± 6.33	36.0 ± 6.86
VF (l)	4.4 ± 2.40	1.9 ± 1.57°	3.5 ± 1.51	1.5 ± 0.48°°°
Blood Pressure				
Systolic (mmHg)	122.1 ± 13.19	119.4 ± 8.03	129.9 ± 17.75	121.6 ± 14.18
Diastolic (mmHg)	82.1 ± 7.27	83.2 ± 9.38	83.9 ± 9.55	81.0 ± 7.93

All data presented as mean ± standard deviation. ° indicates statistical significance; ° P <0.05, °° P <0.01, °°° P <0.001, difference between timepoints within the genotype group. † represents significant difference in baseline values across groups and end intervention values across groups; P<0.05, ††P<0.01.

As displayed in **Table 6.8**, carriers of the A* allele (AA and GA) of TNF-α – 308 had significantly higher levels of inflammatory markers TNF-α (p =0.009) and leptin (p =0.001) than GG homozygotes. Carriers of the A* allele also had significantly higher IHL content (p=0.033), HOMA-IR (p =0.014), weight (p =0.007), BMI (p =0.024) and FM (p =0.023) than GG homozygotes.

Similar to results for SNP TNF-α – 308 at baseline, serum levels of inflammatory markers TNF-α and leptin were significantly higher (p <0.01) in carriers of the A* allele compared to GG homozygotes following dietary intervention.

At 12-weeks, improvements in the level of circulating adiponectin (p =0.042) and triglycerides (p =0.05), VF (p <0.001) and systolic blood pressure (p =0.037) were observed in GG homozygotes of SNP TNF-α – 308. Serum concentrations of IL-6 increased significantly in GG homozygotes (p =0.032), whereas no change was observed in carriers of the A* allele. Furthermore, serum

concentrations of fasting glucose (p =0.037) and HOMA-IR (p =0.040) significantly decreased in carriers of the A* allele, however these effects weren't observed in GG homozygotes.

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At baseline (*n = 42*), there was a moderate, positive correlation observed between body weight (kg), BMI, and fat mass (%) and SNP TNF-α -308, which was statistically significant (r_{pb} *= 0.408, p = 0.007,* r_{pb} *= 0.348, p = 0.024, and* r_{pb} *= 0.356, p = 0.021, respectively*). At end intervention, there was a moderate, positive correlation observed between serum TNF-α and SNP TNF-α -308, which was statistically significant (r_{pb} *= 0.493, n = 39, p = 0.001*) and a moderate, positive correlation observed between waist circumference and SNP TNF-α -308, which was statistically significant (r_{pb} *= 0.321, n = 39, p = 0.046*). Results are presented in **Appendix 11; Supplementary Table 8,** represented as a box plot which displays the distribution of variables between risk and non-risk genotypes.

Table 6.8. Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to TNF-α -308A/G genotype

	TNF-α -308A/G			
	***C**arriers of the A allele**		*GG homozygotes*	
	Baseline (n = 10)	End Int (n = 8)	Baseline (n = 32)	End Int (n = 31)
Inflammatory Markers				
hs-CRP (mg/L)	4.6 ± 3.07	3.4 ± 2.37	2.9 ± 2.33	2.8 ± 2.43
TNF-alpha (pg/mL)	8.7 ± 8.93††	6.2 ± 1.65††	3.8 ± 1.55	3.8 ± 1.75
IL-6 (pg/mL)	8.6 ± 10.95	8.4 ± 8.78	12.0 ± 19.87	15.2 ± 22.14°
Adiponectin (µg/mL)	21.4 ± 14.51	20.3 ± 10.58	13.7 ± 11.03	17.9 ± 18.95°
Leptin (ng/mL)	27.5 ± 12.25††	24.3 ± 12.41††	12.6 ± 9.79	13.0 ± 10.64
Resistin (ng/mL)	44.1 ± 14.93	43.8 ± 11.54	38.3 ± 19.81	38.3 ± 19.67
Liver Outcomes				
IHL (%)	21.6 ± 10.55†	17.7 ± 11.74	9.1 ± 7.79	9.1 ± 9.75
LSM (kPa)	13.3 ± 15.45	10.9 ± 8.36	9.0 ± 9.31	8.8 ± 8.28
Biochemistry				
Glucose (mmol/L)	7.1 ± 2.22	6.6 ± 1.66°	6.1 ± 1.70	6.2 ± 2.05
Insulin (mIU/L)	25.0 ± 12.83	21.8 ± 13.98	16.3 ± 9.67	14.5 ± 8.35
HOMA-IR	8.6 ± 6.85†	7.0 ± 6.39°	4.6 ± 3.46	4.2 ± 3.60
ALT (U/L)	57.8 ± 33.45	51.8 ± 36.40	58.3 ± 32.17	56.0 ± 31.02
AST (U/L)	39.4 ± 17.60	39.0 ± 25.87	36.6 ± 19.10	36.1 ± 20.86
GGT (U/L)	87.0 ± 70.23	67.8 ± 38.66	117.3 ± 117.47	108.0 ± 87.18
ALP (U/L)	93.4 ± 27.62	99.8 ± 29.08	92.2 ± 31.85	94.3 ± 27.66
Cholesterol (mmol/L)	4.3 ± 1.07	4.1 ± 1.08	5.2 ± 1.63	5.1 ± 1.50
HDL (mmol/L)	1.2 ± 0.24	1.1 ± 0.27	1.2 ± 0.26	1.2 ± 0.27

LDL (mmol/L)	2.5 ± 1.06	2.2 ± 1.09†	3.2 ± 1.34	3.2 ± 1.29
Triglycerides (mmol/L)	1.5 ± 0.51	1.7 ± 0.61	1.9 ± 0.94	1.7 ± 0.76°
Anthropometry				
Weight (kg)	105.2 ± 30.34††	100.1 ± 25.85	83.7 ± 17.41	84.1 ± 17.53
BMI	36.1 ± 8.63†	34.5 ± 6.65	31.0 ± 4.90	30.8 ± 4.51
WC (cm)	118.4 ± 25.39	113.6 ± 18.05	103.6 ± 11.91	102.8 ± 11.81
FM (%)	45.0 ± 6.95†	43.2 ± 6.90	38.5 ± 7.54	37.8 ± 7.60
VF (l)	4.6 ± 2.97	2.2 ± 2.10	3.6 ± 1.49	1.5 ± 0.57°°°
Blood Pressure				
Systolic (mmHg)	117.9 ± 13.58	120.3 ± 11.66	129.2 ± 16.26	120.9 ± 12.47°
Diastolic (mmHg)	81.5 ± 8.37	84.6 ± 10.94	83.6 ± 8.74	81.1 ± 7.68

All data presented as mean ± standard deviation. ° indicates statistical significance; ° P <0.05, °° P <0.01, °°° P <0.001, difference between timepoints within the genotype group. † represents significant difference in baseline values across groups and end intervention values across groups; P<0.05, ††P<0.01.

Carriers of the T allele (TT and GT) of adiponectin +276 had significantly higher levels of fasting glucose (p =0.023) than GG* homozygotes, as displayed in **Table 6.9**. Conversely, T allele carriers had significantly lower concentrations of cholesterol (p =0.035) and systolic blood pressure measurements (p =0.023) than GG* homozygotes.

Following dietary intervention, serum concentrations of adiponectin significantly increased (p =0.049) and systolic blood pressure significantly decreased (p =0.009) in GG* homozygotes at SNP adiponectin +276, whereas these changes were not observed in carriers of the T allele. Conversely, LSM (kPa) decreased significantly in carriers of the T allele (p =0.047), whereas no effect was observed in GG* homozygotes. There were significant reductions in VF for both carriers of the T allele (p <0.001) and GG* homozygotes (p =0.002) at SNP adiponectin +276.

At 12-weeks, serum concentrations of total cholesterol (p =0.019) and HDL-cholesterol (p =0.047) were significantly lower in carriers of the T allele compared with GG* homozygotes.

Table 6.9. Circulating inflammatory markers, liver outcomes, biochemistry and anthropometry at baseline and after 12-weeks of dietary intervention according to adiponectin +276G/T genotype

	Adiponectin +276G/T			
	Carriers of the T allele		*GG homozygotes**	
	Baseline (n = 18)	End Int (n = 15)	Baseline (n = 24)	End Int (n = 24)
Inflammatory Markers				
hs-CRP (mg/L)	3.9 ± 3.02	3.6 ± 2.41	2.8 ± 2.13	2.5 ± 2.34
TNF-alpha (pg/mL)	5.6 ± 7.23	4.3 ± 2.37	4.6 ± 1.82	4.3 ± 1.73

IL-6 (pg/mL)	8.0 ± 10.21	9.9 ± 10.05	13.7 ± 22.27	16.2 ± 24.45
Adiponectin (µg/mL)	12.1 ± 9.26	12.4 ± 8.50†	18.3 ± 13.73	22.1 ± 20.56°
Leptin (ng/mL)	16.1 ± 12.91	12.9 ± 11.73	16.4 ± 11.81	16.8 ± 11.85
Resistin (ng/mL)	43.2 ± 24.07	43.3 ± 21.42	37.0 ± 13.14	37.0 ± 16.09
Liver Outcomes				
IHL (%)	11.3 ± 9.18	10.3 ± 12.42	11.4 ± 9.96	10.5 ± 9.46
LSM (kPa)	14.2 ± 15.56	12.7 ± 11.31°	6.8 ± 3.67	7.2 ± 4.82
Biochemistry				
Glucose (mmol/L)	6.8 ± 2.17†	7.2 ± 2.69	6.0 ± 1.54	5.7 ± 1.06
Insulin (mIU/L)	20.7 ± 12.65	17.0 ± 11.82	16.7 ± 9.48	15.4 ± 8.88
HOMA-IR	6.7 ± 5.64	6.1 ± 6.21	4.7 ± 3.81	3.9 ± 2.47
ALT (U/L)	54.4 ± 27.56	51.0 ± 26.92	60.9 ± 35.39	57.7 ± 34.70
AST (U/L)	37.8 ± 16.64	36.2 ± 19.56	36.9 ± 20.26	37.0 ± 23.26
GGT (U/L)	98.8 ± 68.88	84.4 ± 38.90	118.6 ± 130.97	109.3 ± 98.22
ALP (U/L)	88.5 ± 21.40	95.0 ± 26.84	95.4 ± 36.14	95.7 ± 28.72
Cholesterol (mmol/L)	4.5 ± 1.72†	4.4 ± 1.56†	5.3 ± 1.35	5.2 ± 1.36
HDL (mmol/L)	1.1 ± 0.22	1.1 ± 0.20†	1.2 ± 0.27	1.3 ± 0.29
LDL (mmol/L)	2.8 ± 1.52	2.6 ± 1.42	3.2 ± 1.12	3.2 ± 1.19
Triglycerides (mmol/L)	1.6 ± 0.84	1.6 ± 0.80	1.9 ± 0.89	1.7 ± 0.69
Anthropometry				
Weight (kg)	88.9 ± 25.70	86.6 ± 21.09	88.9 ± 20.83	87.9 ± 20.09
BMI	32.6 ± 7.05	31.2 ± 5.02	32.0 ± 5.75	31.8 ± 5.32
WC (cm)	107.6 ± 20.42	104.2 ± 14.78	106.7 ± 14.43	105.5 ± 13.41
FM (%)	40.3 ± 7.50	37.7 ± 7.30	39.9 ± 8.23	39.6 ± 7.99
VF (l)	4.3 ± 2.32	1.4 ± 0.47°°°	3.5 ± 1.57	1.8 ± 1.31°°
Blood Pressure				
Systolic (mmHg)	123.1 ± 20.72†	121.1 ± 17.32	129.1 ± 11.74	120.5 ± 7.25°°
Diastolic (mmHg)	79.6 ± 8.73	81.1 ± 9.24	85.8 ± 7.66	82.4 ± 8.10

All data presented as mean ± standard deviation. ° indicates statistical significance; ° P <0.05, °° P <0.01, °°° P <0.001, difference between timepoints within the genotype group. † represents significant difference in baseline values across groups and end intervention values across groups; P<0.05, ††P<0.01.

6.4.6 The influence of inflammatory gene variants' on circulating inflammatory markers in a NAFLD cohort

Linear regression analysis was carried out to determine if the CRP +1846 variant predicts serum hs-CRP (**Table 6.10**) in this NAFLD cohort after the dietary intervention period. The model controlled for age, sex, BMI and baseline levels of serum hs-CRP. The CRP +1846 variant was

entered as a categorical variable with genotype TT/TC as the reference or non-risk variable (coded as 0.00), and CC as risk variable (coded as 1.00).

The CRP +1846 variant did not significantly predict serum levels of hs-CRP when controlling for age, sex, BMI and baseline levels of serum hs-CRP (β = -0.211, p = 0.056). This result was approaching statistical significance and the negative standardised (β) regression coefficient indicates that the risk (CC) genotype predicts significant reductions in serum CRP levels after the dietary intervention period when compared to the non-risk (TT/TC) genotype. Age, sex and BMI variables were not significant individual predictors in the model for hs-CRP (p>0.05). As expected, baseline hs-CRP level was the only variable in the model which significantly predicted hs-CRP following dietary intervention (β = 0.839, p <0.001).

Linear regression analysis was carried out to determine if IL-6 -174G/C variant predicts serum IL-6 concentration (**Table 6.10**) after a 12-week dietary intervention. The model controlled for age, sex, BMI and baseline levels of serum IL-6. The IL-6 -174G/C variant was entered as a categorical variable with genotype GG as the reference or non-risk variable (coded as 0.00), and CG/CC as risk variable (coded as 1.00). The IL-6 -174G/C variant did not significantly predict serum levels of IL-6 at the post intervention stage when controlling for age, sex, BMI and IL-6 at baseline (β = 0.082, p = 0.493). Age, sex and BMI variables were not significant individual predictors in the model for hs-CRP (p>0.05). Baseline IL-6 was the only variable in the model which significantly predicted IL-6 following dietary intervention (β = 0.775, p <0.001).

Linear regression analysis was carried out to determine if the TNF-α -308 variant predicts serum levels of TNF-α (**Table 6.10**) after a 12-week dietary intervention, during which participants improved their diet quality. The model controlled for age, sex, BMI and baseline levels of serum TNF-α. The TNF-α -308 variant was entered as a categorical variable with genotype GG as the reference or non-risk variable (coded as 0.00), and AG/AA as risk variable (coded as 1.00). In this regression model, the total variance explained by the variables was 50%, R^2 = 0.497, F (5, 32) = 6.33, p = 0.001. Step 1 of the model which adjusted for age, sex, BMI and TNF-α at baseline accounted for 41% of the variance in TNF-α, R^2 = 0.41, F (4 33) = 5.61, p = 0.001. The addition of the TNF-α – 308 variant to the regression model (step 2) accounted for an additional and significant 9% variance in hs-CRP, ΔR^2= 0.090, ΔF (1, 32) = 5.88, p = 0.021. The TNF-α -308 variant significantly predicted serum levels of TNF-α when controlling for age, sex, BMI and baseline levels of TNF-α (β = 0.356, p = 0.021). The positive standardised (β) regression coefficient indicates that the risk (AA/AG) genotype prediczts significant increases in serum TNF-α when compared to the non-risk (GG) genotype. Baseline level of TNF-α was the strongest predictor of serum TNF-α after dietary intervention (β = 0.556, *p = 0.001),* followed by the TNF-α – 308 variant as the next strongest predictor of TNF-α.

Linear regression analysis was carried out to determine if the adiponectin +276G/T variant predicts serum adiponectin concentration (**Table 6.10**) after 12-weeks of dietary intervention. The analysis was carried out controlling for age, sex, BMI and baseline level of adiponectin. The +276G/T variant was entered as a categorical variable with genotype TT/TC as the reference or non-risk variable (coded as 0.00), and GG as risk variable (coded as 1.00). The +276G/T variant did not significantly predict serum level of adiponectin when controlling for age, sex, BMI and baseline adiponectin ($\beta = -0.129$, $p = 0.221$). BMI ($\beta = 0.256$, $p = 0.022$) and baseline adiponectin ($\beta = 0.608$, $p < 0.001$) were significant predictors of adiponectin following dietary intervention.

Table 6.10. Multiple linear regression models for the outcome variables of inflammatory markers hs-CRP, IL-6, TNF-α and adiponectin

Outcome Variable	R	R^2	Adj. R^2	P	Predictor Variable	Standardised Beta (β)	P	95% CI
hs-CRP (mg/L)	0.84	0.70	0.65	0.000*	Age (y)	0.012	0.922	-0.04, 0.05
					Sex	-0.090	0.458	-1.62, 0.75
					BMI	0.080	0.539	-0.07, 0.13
					Baseline hs-CRP (mg/L)	0.839	0.000*	0.52, 1.05
					CRP +1846 Variant	-0.211	0.056	-2.14, 0.14
IL-6 (pg/mL)	0.78	0.61	0.55	0.000*	Age (y)	-0.091	0.458	-0.02, 0.01
					Sex	0.108	0.391	-0.19, 0.47
					BMI	0.177	0.150	-0.01, 0.04
					Baseline IL-6 (pg/mL)	0.775	0.000*	0.02, 0.04
					IL-6 -174 Variant	0.082	0.493	-0.20, 0.42
TNF-α (pg/mL)	0.71	0.50	0.42	0.000*	Age (y)	-0.060	0.673	-0.05, 0.04
					Sex	0.010	0.944	-1.12, 1.20
					BMI	-0.278	0.053	-0.18, 0.01
					Baseline TNF-α (pg/mL)	0.556	0.001*	0.10, 0.34
					TNF-α -308 Variant	0.356	0.021*	0.26, 2.99
Adiponectin (µg/mL)	0.83	0.69	0.64	0.000*	Age (y)	0.165	0.139	-0.08, 0.53
					Sex	0.094	0.394	-4.49, 11.13
					BMI	0.256	0.022*	0.11, 1.32
					Baseline adiponectin (µg/mL)	0.608	0.000*	0.54, 1.19
					Adiponectin +276 Variant	0.129	0.221	-2.84, 11.83

*Significant, P<0.05.

6.4.7 Genotype Nutrient Interactions

To investigate the presence of SNP*nutrient interactions in association with the serum inflammatory marker, a Univariate General Linear Model was used. Macronutrients were dichotomized by the median level of nutrient intake variable at end intervention (i.e., above and below the median). Change in inflammatory marker was calculated as the difference between pre- and post-intervention values. Model 1 of each two-way ANOVA analysis represents the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates. Model 2 of each two-way ANOVA analysis represents the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (at baseline).

No significant nutrient–genotype interaction effect was observed for CRP +1846, IL-6 -174 and adiponectin +276 polymorphisms in association with circulating inflammatory markers (P for interaction >0.05) (data not shown).

Several gene-nutrient interactions were observed for TNF-α -308, in association with changes in serum TNF-α concentration. There was a significant interaction found between energy intake and TNF-α -308 variant for serum TNF-α levels in ANOVA model 1 and model 2 ($P = 0.031$ and $P = 0.047$, respectively).

In **Figure 6.2**, the effect of carrying an A allele in TNF-α -308 results in greater reductions in serum TNF-α with energy intake above the median of 6,885kJ (AG/AA: -8.11 ± 2.1 vs GG: -0.97 ± 1.6 pg/mL , P = 0.02). This difference remained significant when the model was adjusted for age, sex and BMI (AG/AA: -7.6 ± 2.3 vs GG: +0.24 ± 1.8 pg/mL, P = 0.02), represented in **Figure 6.2.**

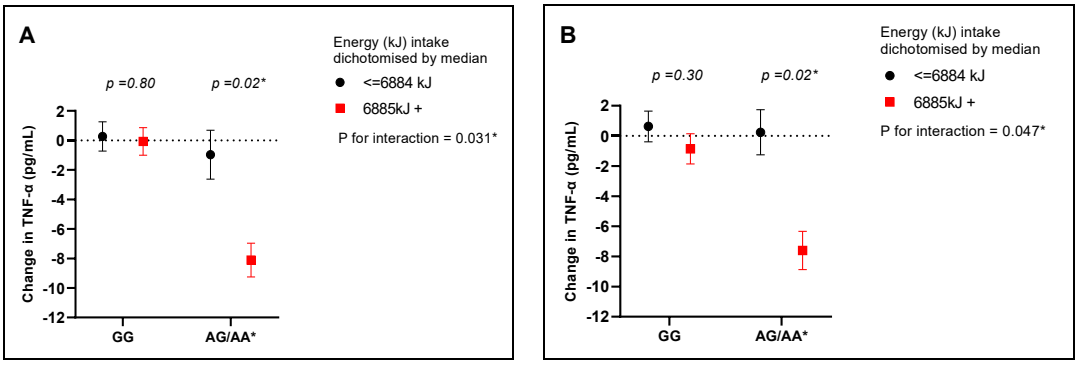


Figure 6.2. Effect of the TNF-α -308A/G single nucleotide polymorphism and energy intake (kJ) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

A significant interaction was found between protein intake (%E) and TNF-α -308 variant for serum TNF-α levels in ANOVA model 1 and model 2 ($P = 0.02$ and $P = 0.03$, respectively).

Figure 6.3 displays a greater reduction in serum TNF-α levels in carriers of the A allele with protein intake above the median of 19.86%E (AG/AA: -7.06 ± 1.8 vs GG: -0.24 ± 1.8 pg/mL, P = 0.04). This difference did not remain significant when the model was adjusted for age, sex and BMI (**Figure 6.3**).

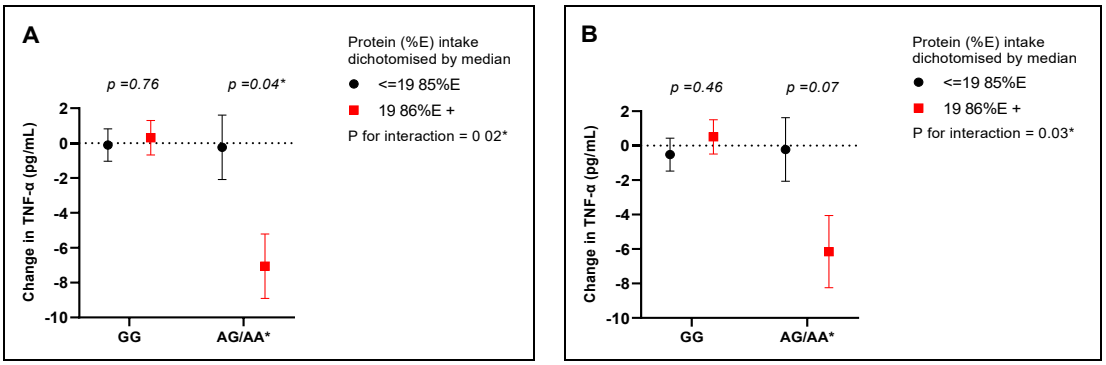


Figure 6.3. Effect of the TNF-α -308A/G single nucleotide polymorphism and protein intake (as percentage contribution to total energy intake) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

The interaction for carbohydrate intake and TNF-α -308 variant in association with serum TNF-α levels was significant in ANOVA model 1 and model 2 ($P = 0.001$ and $P = 0.001$, respectively).

In **Figure 6.4**, the effect of carrying the A allele in TNF-α -308 results in a greater reduction in circulating TNF-α with lower carbohydrate intake of below the median of ≤37.93%E (AG/AA: -13.81 ± 2.1 vs GG: -0.27 ± 1.2 pg/mL, P = 0.001). In **Figure 6.4**, this result remained significant when adjusting for age, sex and BMI (AG/AA: -14.43 ± 2.6 vs GG: -0.29 ± 1.2 pg/mL, P = 0.001).

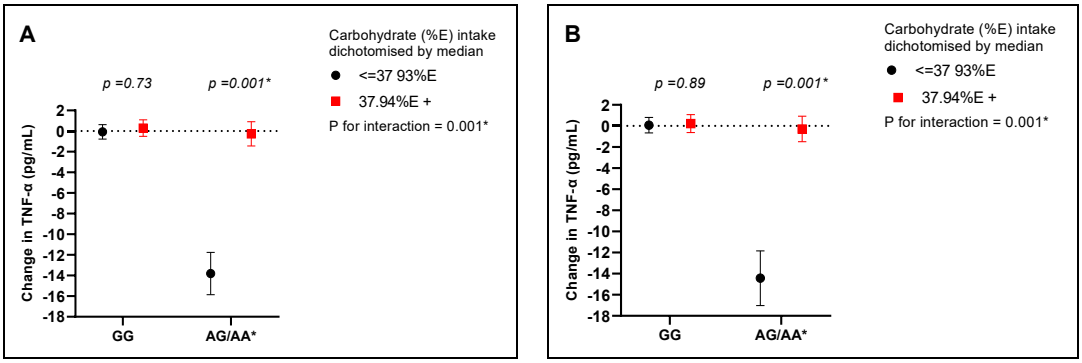


Figure 6.4. Effect of the TNF-α -308A/G single nucleotide polymorphism and carbohydrate intake (as percentage contribution to total energy intake) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as

group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

Gene-nutrient interactions for total sugar intake and TNF-α -308 variant in association with serum TNF-α levels was significant in ANOVA model 1 and model 2 (P = 0.02 and P = 0.04, respectively).

Figure 6.5 demonstrates the effect of carrying the A allele in TNF-α -308, which results in larger reductions in serum TNF-α with lower sugar intake below the median of ≤14.73%E (AG/AA: -8.11 ± 2.1 vs GG: -0.97 ± 1.7 pg/mL, P = 0.04). This result remained significant in ANOVA model 2, which controlled for age, sex and BMI (AG/AA: -7.32 ± 2.3 vs GG: -0.15 ± 1.8 pg/mL, P = 0.05).

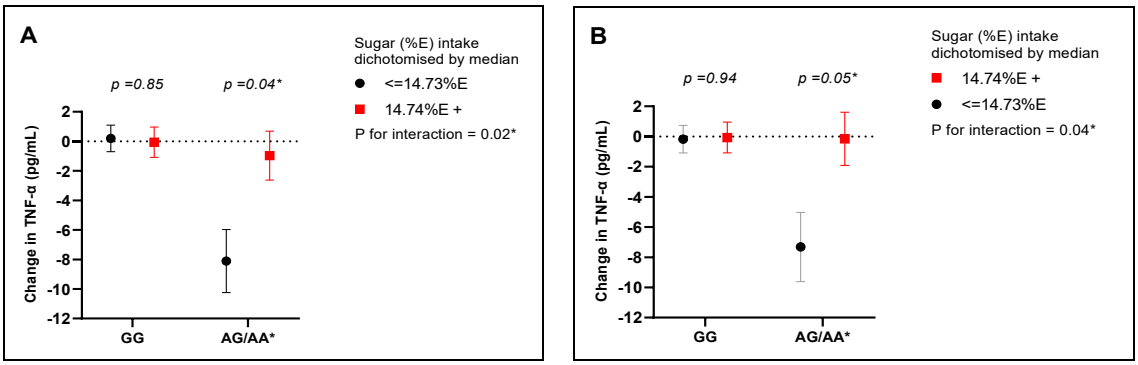


Figure 6.5. Effect of the TNF-α -308A/G single nucleotide polymorphism and total sugar intake (as percentage contribution to total energy intake) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

A significant interaction was found between MUFA (%E) and TNF-α -308 variant for serum TNF-α levels in ANOVA model 1 and model 2 (*P* = 0.007 and *P* = 0.05, respectively).

Figure 6.6 displays a greater reduction in serum TNF-α levels in carriers of the A allele with MUFA intake above the median of 16.83%E (AG/AA: -8.97 ± 2.0 vs GG: -0.46 ± 1.6 pg/mL, P = 0.01) and **Figure 6.6** displays the significant effect remained significant after controlling for age, sex and BMI (AG/AA: -7.79 ± 2.5 vs GG: -0.68 ± 1.6 pg/mL, P = 0.05).

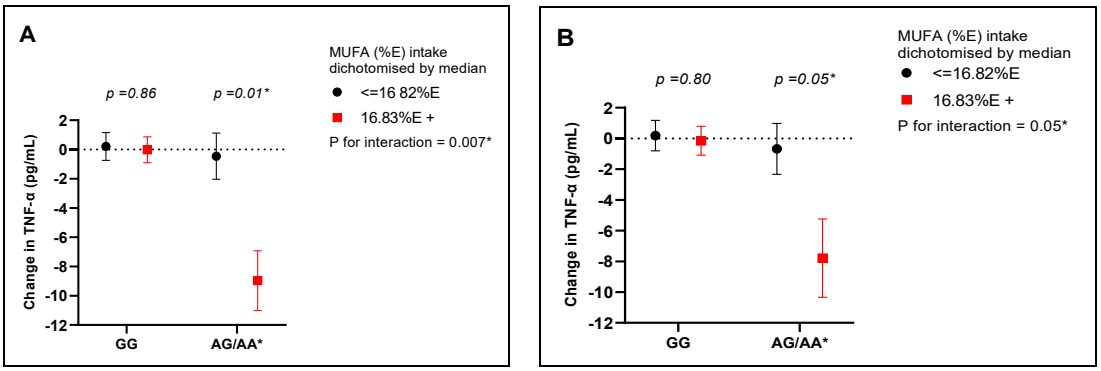


Figure 6.6. Effect of the TNF-α -308A/G single nucleotide polymorphism and monounsaturated fatty acid intake (as percentage contribution to total energy intake) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

A significant interaction between total fat intake and circulating levels of serum TNF-α was observed for the TNF- α -308 variant for ANOVA model 2 (P = 0.041)which is presented in **Figure 6.7**, but not ANOVA model 1 (P = 0.281) (data not shown).

In **Figure 6.7**, after controlling for age, sex and BMI, the effect of carrying an A allele in TNF-α -308 results in greater reductions in serum TNF-α with fat intake below the median of 37.92%E (-4.27 ± 1.5 pg/mL) and increases in serum TNF-α with fat intake above the median of 37.93%E (2.47 ± 2.9 pg/mL), though differences were not significant.

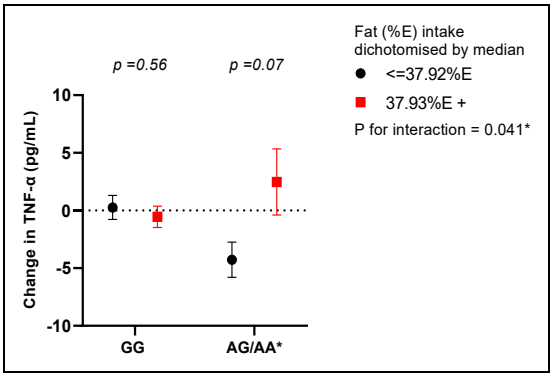


Figure 6.7. Effect of the TNF-α -308A/G single nucleotide polymorphism and fat intake (as percentage contribution to total energy intake) on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis, representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

6.4.8 Dietary Adherence: Genotype x MedDiet Interactions

To investigate the presence of SNP*MedDiet interactions in association with the serum inflammatory markers, a Univariate General Linear Model was used. Each SNP was investigated with low to moderate versus moderate to high MedDiet adherence based on PREDIMED MedDiet scores at the end of the dietary intervention in two-way ANOVA models. Low to moderate adherence was classified as a score of 4.00-7.65 and moderate to high adherence was classified as 7.66-14.00. These cut-off values represent the overall group scores below and above the mean score for MedDiet adherence at the end intervention timepoint. The analysis of the diet–genotype interaction on inflammatory markers was calculated using the difference between post- and pre-intervention data of inflammatory marker variables for ANOVA.

No diet–genotype interaction effect was observed for any of the analysed inflammatory markers (P for interaction >0.05) (data shown below). Model 1 of each two-way ANOVA analysis represents the diet-genotype interaction for change in inflammatory marker without the inclusion of any covariates. Model 2 of each two-way ANOVA analysis represents the diet-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (at baseline).

6.4.8.1 CRP

There was no significant interaction found between level of MedDiet adherence and CRP +1846 variant for serum hs-CRP levels for ANOVA model 1 or model 2 (P = 0.523 and P = 0.764, respectively)**(Figure 6.8)**. SNP CRP +1846 did not show an independent role on the magnitude of change in hs-CRP concentrations from pre- to post-intervention, with level of adherence to the MedDiet. There was, however, a borderline level of significance observed for mean change in hs-CRP concentration between non-risk and risk genotypes in the low diet adherence group whereby non-risk genotypes had greater increases in hs-CRP than risk genotypes which experienced reductions in hs-CRP (TT/TC: +1.05±0.47 vs CC: -0.42±0.55 mg/L, P = 0.06). This result was maintained when the model was adjusted for age, sex and BMI (TT/TC: 0.98±0.51 vs CC: -0.6±0.57 mg/L P = 0.06).

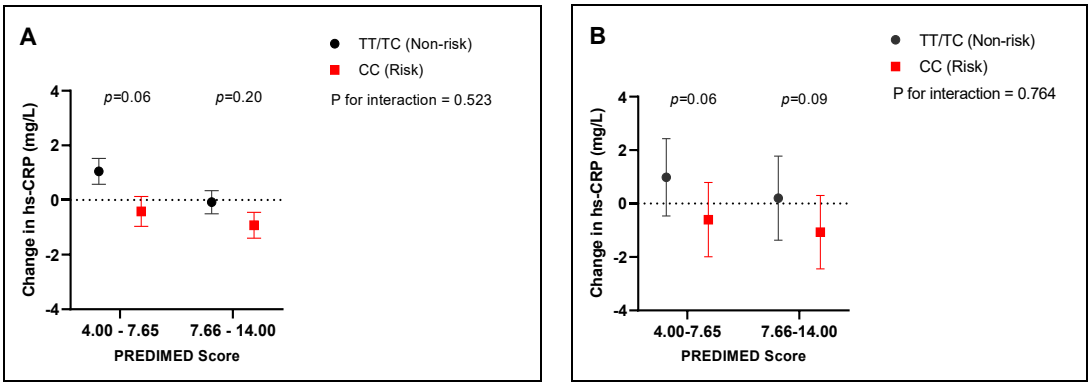


Figure 6.8. Effect of the CRP +1846C/T single nucleotide polymorphism and adherence to the Mediterranean Diet measured using the Prevención con Dieta Mediterránea; Prevention with the Mediterranean Diet (PREDIMED) score on change in high-sensitivity C-reactive protein (hs-CRP) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

6.4.8.2 TNF-α

There was no significant interaction found between level of MedDiet adherence and TNF-α -308 variant for serum TNF-α levels for ANOVA model 1 or model 2 (P = 0.294 and P = 0.078, respectively)**(Figure 6.9)**. In the low MedDiet adherence group mean change in serum TNF-α differed significantly between non-risk and risk genotypes for SNP TNF-α -308 (GG: 0.15±1.2 vs AG/AA: -4.58±1.63 pg/mL, P = 0.03). No significant difference was observed between non-risk and risk genotypes when the model was adjusted for age, sex and BMI (GG: 0.27±1.19 vs AG/AA: -4.28±1.53 pg/mL, P = 0.06), although the magnitude of change in serum TNF-α remained vastly different for low diet adherers.

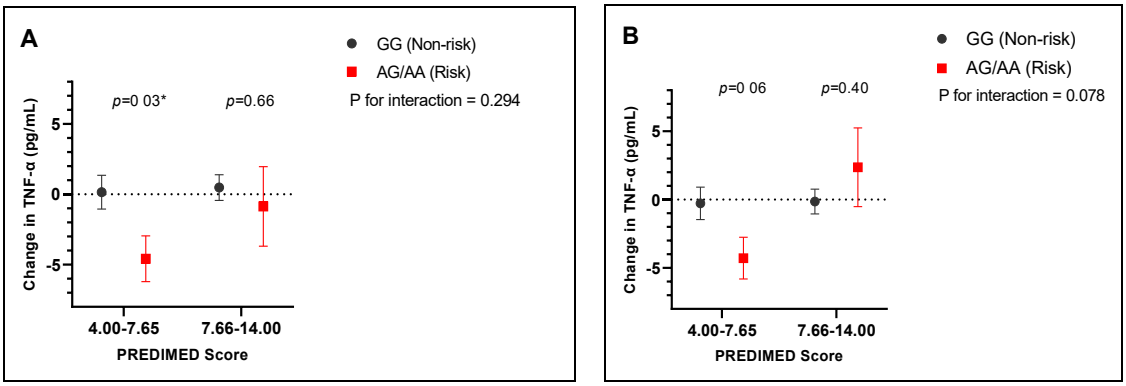


Figure 6.9. Effect of the TNF-α -308A/G single nucleotide polymorphism and adherence to the Mediterranean Diet measured using the Prevención con Dieta Mediterránea; Prevention with the Mediterranean Diet (PREDIMED) score on change in tumor necrosis factor-alpha (TNF-α) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of

any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

6.4.8.3 IL-6

There was no significant interaction found between level of MedDiet adherence and IL-6 -174 variant for serum IL-6 levels for ANOVA model 1 or model 2 (P = 0.326 and P = 0.384, respectively)**(Figure 6.10)**. The effect of dietary adherence on serum IL-6 concentration did not differ significantly (P>0.05) for risk vs non-risk genotypes of SNP IL-6 -174.

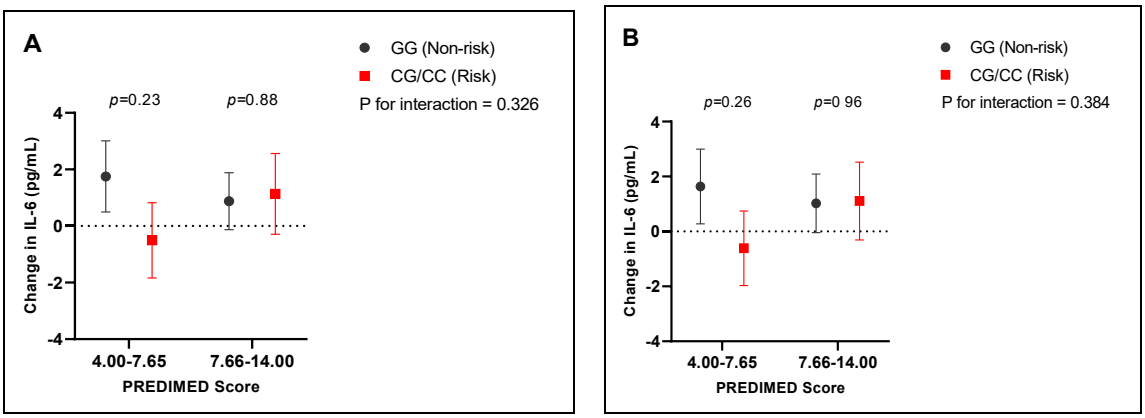


Figure 6.10. Effect of the IL-6 -174G/C single nucleotide polymorphism and adherence to the Mediterranean Diet measured using the Prevención con Dieta Mediterránea; Prevention with the Mediterranean Diet (PREDIMED) score on change in interleukin-6 (IL-6) concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

6.4.8.4 Adiponectin

There was no significant interaction found between level of MedDiet adherence and adiponectin +276 variant for serum adiponectin levels for ANOVA model 1 or model 2 (P = 0.960 and P = 0.957, respectively)**(Figure 6.11)**. The effect of dietary adherence on serum adiponectin concentration did not differ significantly (P>0.05) for risk vs non-risk genotypes of SNP adiponectin +276.

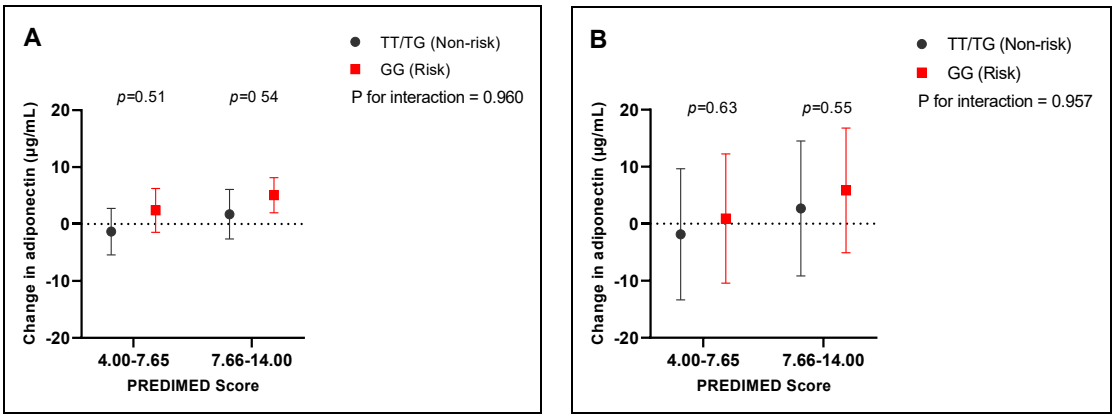


Figure 6.11. Effect of the adiponectin +276G/T single nucleotide polymorphism and adherence to the Mediterranean Diet measured using the Prevención con Dieta Mediterránea; Prevention with the Mediterranean Diet (PREDIMED) score on change in adiponectin concentration between baseline (0-weeks) and end of diet intervention (12-weeks) in a two-way ANOVA analysis representing the nutrient–genotype interaction for change in inflammatory marker without the inclusion of any covariates (A) and a two-way ANOVA analysis representing the nutrient-genotype interaction for change in inflammatory marker controlling for age, sex and BMI (B). Values are expressed as group means ± SDs. Symbol (*) is used for comparisons between intake of diet variable above and below the median with genotype groups, p ≤0.05 represents statistical significance.

6.5 Discussion

The results from this explorative study add to existing literature that identifies the prevalence of CRP +184, IL-6 -174, TNF-α -308 and adiponectin +276 polymorphisms in patients diagnosed with NAFLD, and suggests that these SNPs have varying impact on inflammatory, liver and metabolic risk markers between risk and non-risk genotypes. This study identified associations between SNPs IL-6 -174 and adiponectin +276 with risk of the MetS and T2DM, respectively, and determined significant gene-nutrient interactions in SNP TNF-α -308 found to influence inflammation, consistent with findings previously reported in the literature. In this group of individuals diagnosed with NAFLD, the majority of participants (91%) had the risk allele (C) for gene variant CRP +1846. Similarly, most participants in this group (90%) had the risk allele (G) for adiponectin +276. For SNP IL-6 -174, although the majority did not carry the risk allele C, many participants (57%) were GG homozygotes which is classified as the high producer phenotype for IL-6. In this study group, only 23% carried the risk allele for the TNF-α -308 polymorphism.

The findings of the present study support the hypothesis that the risk allele of SNP TNF-α -308 and IL-6 –174 may influence differences in inflammatory, liver and metabolic risk markers in comparison to non-risk alleles and may predict level of serum inflammatory markers and responsiveness to dietary change. Carriers of the risk (A) allele displayed significantly higher levels of serum TNF-α and insulin resistance at both timepoints and were found to predict significant increases in serum TNF-α, compared to the non-risk (GG) genotype, after dietary intervention. The

first *in vitro* study to show that the rare allele at TNF2 (-308A) which lies on the extended haplotype HLA-A1-B8-DR3DQ2 was associated with high production and circulating TNF-α was published in 1993 by Wilson and colleagues.[675] After which several *in vitro* studies confirmed this association,[640, 643, 676] and *in vivo* studies have continued to observe significantly higher circulating TNF-α in TNF2 carriers with NAFLD and/or NASH.[668, 677] The TNF2 gene variant has also been associated with increased risk for HCC, higher risk for hepatic fibrosis and more severe liver damage in pairs of sex-matched and age-matched patients with HCC versus unrelated healthy controls.[678] Another study found an association between the TNF2 variant and increased risk for developing obesity in patients with the MetS compared to healthy controls, as well as significantly higher systolic arterial blood pressure and plasma insulin levels in carriers of the TNF2 allele who had the MetS.[667] Such findings support the hypothesis that the TNF gene is involved in the pathogenesis of inflammatory-mediated diseases and the contributory effects of the TNF2 gene variant to increasing cytokine secretion and unbalance.[667] Conversely, a number of case-control studies have reported no significant difference in the genotypic distributions and the allelic frequency of TNF2 between NAFLD and control groups.[78, 679-681] The present study adds to the existing literature on TNF-α-308 in NAFLD, though larger groups of patients with NAFLD compared with healthy controls are required to confirm these findings and explore the effects of the polymorphism on TNF-α expression.

A major finding of this study was the significant gene-nutrient interactions that were observed between risk genotypes of the TNF-α -308 SNP with total energy, protein, carbohydrate, sugar, total fat and monounsaturated fat intake. More specifically, the TNF2 (-308A) allele was associated with energy (kJ) and protein (percentage derived from energy) intake levels above the median, that was in turn associated with a decrease in the level of circulating TNF-α. Carbohydrate and sugar intake (both percentage derived from energy intake) below the median levels were also associated with decreased levels of circulating TNF-α. Alternatively, a gene-nutrient interaction was found between the TNF2 allele and MUFA (percentage derived from energy) intake above the median associated with reductions in circulating TNF-α. Moreover, total fat intake (percentage derived from energy) was associated with circulating TNF-α to the effect of a reduction in the cytokine below median intakes and increases in the cytokine above median intake. Several studies of gene expression *in vitro*, animal and human models have investigated the effect of dietary fat on TNF-α concentrations.[682, 683] The basis of these investigations was the known role TNF-α plays in regulating not only the inflammatory response, but also in regulating lipid metabolism pathways in different cells, tissues and organs. TNF-α promotes dyslipidemia by inducing lipolysis and stimulation of free fatty acid (FFA) release.[683] Increased TNF-α concentrations have been reported in clinical patients with dyslipidemia compared with healthy subjects,[684] however blockading TNF-α production tends to improve lipid metabolism.[685] Studies in rodent models identified that TNF gene expression varies in response to a high-fat diet versus one that is supplemented with omega-3 (n-3) polyunsaturated fatty acid (PUFA).[686, 687] In mice, supplementing the diet with n-3 PUFA

decreased TNF-α gene expression[686] and in rats the high fat diet alone increased both body weight and fat mass, whereas the n-3 PUFA supplemented diet resulted in less weight gain, decreased food intake and increased leptin production.[687] Gene expression of TNF-α was also increased by the high fat diet alone but did not change the rats supplemented with n-3 PUFA.[687] In humans, similar effects were seen in multiple studies for n-3 PUFA intake but not for MUFA intake.[683] There is an independent association between dietary fatty acids on TNF-α expression, which may be influenced by individual variability of the TNF gene.

The TNF-α –308 SNPs has been shown to modulate the relationship between dietary fat intake on serum lipid profiles in different populations. Fontaine-Bisson et al. (2007) found that PUFA intake was inversely associated with high-density lipoprotein-cholesterol (HDL-c) concentrations in TNF2 risk allele carriers (–308A) in a group of ethnically diverse diabetic Canadians.[688] More recently, interactions between dietary fat intake and the TNFA –308 SNP on serum lipid profiles in black and white South African women showed that with increasing dietary fat intake, serum lipids increased in black women with the –308 GA + AA genotypes; however, with increasing n-3 PUFA and ALA intake, total cholesterol: HDL-c ratio (T-C:HDL-c ratio) decreased in black South African women only.[689, 690] Investigators suggest the data indicates that the presence of TNF2 alone does not confer risk but rather may be indicative of a greater responsiveness or sensitivity of an individual to changes in dietary intake.[689, 690] This notion of variant allele responsiveness to change in dietary intake has been discussed extensively in the nutrigenomics literature. To the best of our knowledge, the present study is the first to identify the contributory effects of the TNF-α -308 gene variant and its interaction with dietary fat intake to modulate serum TNF-α levels in a NAFLD population. It is possible that the inherent inflammatory status, potentially due to a pre-existing condition such as NAFLD, could determine the extent of the inflammatory response to different dietary fatty acids. In addition to the independent influence of dietary fatty acids on TNFα production, variation in the TNFA gene may also contribute to the individual variability observed in TNFα production and TNFA gene expression.[689, 690] Indeed, there is a call for further research in this area, noting that the role of gene polymorphisms in the effect of nutrients and dietary patterns on inflammation requires much greater exploration.[629]

Genetic variations in the CRP gene, including the +184 SNP, have also shown gene-diet interactions that can affect blood CRP concentration. In fact, a recent study in the HELENA study population which investigated CRP SNPs (rs3093068, rs1205 and rs1130864) found that a higher MedDiet adherence (determined by the Mediterranean Dietary Score (MDS))[691] and higher fish intake reduced CRP concentrations for homozygous major allele carriers (CC) for the rs3093068 SNP only.[629] Interestingly, reduced CRP was even more pronounced minor allele carriers (CG/GG) which are genetically at higher inflammatory risk.[629] Unfortunately, no such gene-diet interactions were observed in the present study. However, an observation of the present study which is in agreement with other studies found that the minor (T) allele of CRP +184 was associated with

significantly lower levels of CRP.[629, 692] An interesting observation was that following dietary intervention, homozygote major (risk) allele carriers (CC) had significantly reduced CRP concentrations whereas T allele carriers experienced no change. Multiple linear regression analysis was used to investigate whether there was an independent association of the CRP +184 variant and circulating CRP levels after adjustment for age, sex, BMI and baseline levels of serum hs-CRP. Results showed that CC homozygotes were associated with decreasing CRP levels when compared to carriers of the T allele (approaching significance). This is an interesting observation as it seems indicative that while the T minor allele is associated with lower level of CRP at baseline, CC homozygotes are more sensitive to change. Although CRP genotypes may be susceptible to lifestyle stimuli, other studies also have not been able to established the effect of diet quality on the inflammation phenotype of CRP +184.[630]

An important observation of this study was that the G/C and G/C-C/C genotypes of IL-6 –174 increased the likelihood of having the MetS and carriers of the C allele had significantly higher levels of insulin resistance, body weight and BMI than GG homozygotes, a finding which is consistent with the literature. Interestingly, allelic differences identified in previous studies which indicate that G/G and G/C genotypes are 'high-producer' phenotypes and the C/C genotype is a 'low-producer' phenotype[151] reflected the level of circulating IL-6 in C allele carriers and GG homozygotes in this study. In the present study, the minor C allele carriers were grouped for analysis and they had lower IL-6 than GG homozygotes. Although the difference was not significant between groups, it is anticipated that with a larger sample CC homozygotes would have displayed significantly higher levels of IL-6. In addition to these differences in transcriptional activity and expression of the cytokine, studies are also conflicting on the effect of this polymorphism in different ethnic populations. The C allele is known to be associated with increased metabolic risk, insulin resistance (IR) and prevalence of diabetes (two-fold increase),[662] especially in Caucasian populations.[186, 665, 693] In Caucasian men the C allele was also associated with high systolic blood pressure and increased susceptibility to coronary heart disease.[664] However, Asian and African populations are almost monomorphic for the G allele, which is also associated with obesity-related comorbidities and diabetes.[186] The study sample in this population was small, therefore we did not observe a significant difference in ethnicities of participants between groups. There were discrete differences observed between Caucasian/European and Asian/Arab groups, whereby a greater number of Asians and Arabs were GG homozygotes. Nevertheless, the findings of the present study are in accordance with the reported activity of IL-6 as a mediator of both inflammation and insulin resistance which adds to the literature for IL-6 -174 in patients with NAFLD.

Another observation of this study was that the T (minor) allele of adiponectin +276 was associated with lowered risk of T2DM in patients with NAFLD. Interestingly, this protective effect of the T allele for SNP +276 has previously been reported in non-NAFLD populations. Studies of nondiabetic Korean and Japanese men have found the +276 T allele to be protective for T2DM and

in lowering insulin resistance.[656, 670] A meta-analysis investigating three adiponectin SNPs found that the -11377 G allele and +45 G allele were significantly associated with increased risk of CVD, whereas the +276 T allele was associated with a significantly decreased risk.[190] Shin et al. (2006) identified different genotype effects for the +276 SNP in 294 nondiabetic, overweight or obese Korean men and women following a 12-week weight loss intervention. Investigators found that prior to any intervention, GG homozygotes presented with significantly lower adiponectin concentrations and higher insulin resistance compared to carriers of the T allele.[657] Following the intervention adiponectin significantly increased and insulin resistance significantly decreased in GG homozygotes only, changes were not observed in T allele carriers.[657] A similar effect was observed for GG homozygotes in the present study, who tended to respond better to diet, displaying significant improvements in adiponectin concentration after the intervention period. In the present study, GG homozygotes had significantly higher levels of adiponectin and non-significantly lower amounts of insulin resistance than T allele carriers.

In contrast to these findings, recent systematic reviews and meta-analyses have found no significant association between ADIPOQ +276G/T and risk of NAFLD.[654] One study in the Han Chinese population reported a significant association between +276G/T and metabolic risk factors of NAFLD, including obesity, hypertension and high cholesterol, as well as a significantly higher prevalence of genotype TT in those with NAFLD.[694] Whereas another Han Chinese population study found no association between the +276G/T polymorphism and NAFLD.[695] Other studies have reported T allele carriers of SNP +276 to be more prevalent in high cardiometabolic risk populations compared to healthy controls.[646, 652, 657, 694, 696-698] This was owing to decreased adiponectin concentrations, increased visceral adiposity and/or insulin resistance.[646, 652, 657, 694, 696-698] Unlike these studies, Musso et al. (2008) found that nondiabetic, nonobese and normolipidemic individuals who were +276 T allele carriers had significantly higher rates of prevalence αconcentration, visceral adiposity, insulin resistance or diet.[696] They also found that adiponectin SNPs +276 GT and TT genotypes modulated postprandial adiponectin response whereby adiponectin was promptly increased in healthy individuals, but slowly released or even decreased in those with NAFLD/NASH.[696] Consistent with the results from the present study, the meaningfulness of the preliminary findings of intervention studies provide possible implications in the prevention of hepato-metabolic disease, although studies into the mechanisms behind the postprandial responses are needed.[657]

A strength of this study was the comprehensive collection of dietary intake and dietary adherence data through 3-day food diaries at each timepoints and validated PREDIMED questionnaire. Many studies of gene-nutrient interactions rely on 24-hour dietary recall at one timepoint only, therefore this study is more informative and is less subjected to recall bias.[629] It is generally thought that multiple genes, each with modest effects, may underlie multifactorial and polygenic diseases such as NAFLD and its associated risk factors.[330, 683] Another strength of the present study was the

examination of four single nucleotide polymorphism from four genes, each of which had data for the corresponding inflammatory markers. Although this was a strength of the study, it was also a limitation. It is difficult to draw widespread conclusions from candidate gene and allele association studies in which there were no haplotypes of the polymorphisms included. Moreover, this study did not include healthy controls who were genotyped for the same SNPs, so we are unable to compare phenotypic traits of disease with disease-free individuals. Lastly, this study contained a small sample size which limited the power to detect significant interactions for very small effects. This study was not powered to detect a change prior to analysis, as it was intended to be exploratory in nature. The results of this and other similar studies should therefore be confirmed in larger cohorts. The field of nutrigenetics is growing and this study, which explores diet-gene interactions that potentially modulate phenotype in NAFLD, adds to existing literature that may offer opportunities to create personalized dietary recommendations and ultimately reevaluate the criteria used to determine dietary recommendations for individuals and populations. Selecting individuals according to their genetic background and looking at the impact of the intervention in genetically known groups of participants may be applied in diet studies of diseases with chronic underlying low-grade inflammation, such as NAFLD.

In conclusion, this study found that polymorphisms in CRP and TNF genes may predict increases in circulating inflammatory markers following dietary intervention to improve diet quality. Identifying individuals or groups of individuals with enhanced sensitivity to dietary intervention due to genetic variation is important in deciphering disease management and prevention at an individual and at a population level. Gene-nutrient interactions were observed in the TNF-α -308 variant, and IL-6 -174 and adiponectin +276 SNPs may influence susceptibility to the MetS and T2DM. These results may have practical and clinical importance once the findings are confirmed in larger cohorts. Gene-nutrient interactions are an important step in the development of personalized diet recommendations and effective, preventative therapeutic strategies. Future studies should focus on deeper analysis of identified gene-nutrient interactions to explain the underlying molecular mechanisms which govern the interactions, specifically between nutrients and the inflammatory phenotype, in larger populations, ethnic groups and sex-specific investigations.

7 Discussion

7.1 Key Findings

Non-alcoholic fatty liver disease (NAFLD) is the most common form of liver disease worldwide, with prevalence rates increasing in parallel with obesity and type 2 diabetes mellitus (T2DM). Among other factors, poor diet and accumulation of adipose tissue promotes production and release of inflammatory cytokines and adipokines which may emphasise metabolic dysfunction, and drive the pathogenesis of NAFLD and progression to non-alcoholic steatohepatitis (NASH). The Mediterranean Diet (MedDiet) has been recommended in the management of NAFLD due to the proposed cumulative and synergistic anti-inflammatory and anti-oxidant effects of the foods, nutrients and non-nutrients consumed in the dietary pattern. There is limited high-quality evidence investigating the effects of a Mediterranean diet on inflammation in an Australian population with diagnosed NAFLD. This doctoral research aimed to determine whether a 12-week *ad libitum* Mediterranean Diet could improve markers of inflammation in Australian patients diagnosed with NAFLD and this was compared to a low-fat diet, which is the standard diet advised for patients with NAFLD.

At baseline, a cross-sectional analysis of the forty-two free-living multi-ethnic participants enrolled in the MEDINA study found that 60% of participants were female, 93% of participants were overweight or obese, 55% met the NCEP ATP III[402] criteria for the Metabolic Syndrome (MetS) and 43% had diagnosed T2DM. Clinical characteristics and overlap of metabolic disorders in this study group were representative of a wider NAFLD population. As expected, participants with the MetS had significantly higher levels of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) score, a surrogate marker of insulin resistance (IR), indicating the presence of metabolic alterations. Insulin resistance is considered a pathogenic driver of chronic hyperglycaemia, inducing an oxidative stress response which triggers an inflammatory response and leading to cell death.[412] Insulin resistance and inflammation are important clinical features in determining the severity and implications of NAFLD and other cardiometabolic disease. Compared with other NAFLD cohorts, participants in the present study did not have remarkably unfavourable inflammatory or cardiometabolic risk marker profiles as would be expected of patients with NAFLD or NASH attending outpatient liver clinics for treatment. Circulating levels of tumour necrosis factor-alpha (TNF-α) and adiponectin corresponded with classifications ranging between "simple steatosis" and "NAFLD diagnosis".[173] Interleukin-6 (IL-6) was found to be lower in this group when compared to the similar groups of NAFLD patients and healthy controls, whereas resistin and leptin were higher, albeit not significantly, in comparison.[416] Similarly, liver outcome measures indicated that intrahepatic fat and liver stiffness was above the normal range whereas liver enzymes were within normal ranges. This descriptive baseline analysis of the MEDINA study cohort provided

insight into Australian patients diagnosed with NAFLD, indicating that while some metabolic biomarkers, inflammatory markers and liver enzymes were elevated to the upper-limit of healthy or "normal" range, these participants could be classified as metabolically "at-risk" or having mild/moderate steatosis and fibrosis based on liver imaging and stiffness measures. While it is known that obesity-driven metabolic dysfunction and disease overlap occur in the NAFLD population, this chapter provides a novel and comprehensive insight into the inflammatory and metabolic risk marker profile of a real-world study cohort based in metropolitan Melbourne, which has scarcely been reported in the literature.

The habitual diet quality of participants in this cohort at baseline was similar to dietary intake patterns observed in other NAFLD populations. Overall, the group were consuming inadequate levels of total energy, carbohydrates and polyunsaturated fatty acids (PUFA) and higher intakes of total fat, saturated fatty acids (SFA) and total sugar, when compared to the recommended daily intakes (RDI's) for this population. Noteworthy features of this cohort's dietary intake at baseline were fat intake patterns, considering the strong causal link between excess fat consumption, obesity and NAFLD, and excess sugar intake as this contributes to the hepatic and metabolic alterations observed in the pathogenesis of NAFLD. Overall dietary intake of this study group was not significantly unfavourable, however, in that the consumption of key macronutrients and food groups did not vastly exceed or fall short of the recommended ranges. This reiterated earlier stated findings that enrolled participants could be considered metabolically "at-risk" or early stages of NAFLD. This was an unexpected result considering their presentation to metropolitan hospital liver clinics and investigators anticipated more progressed or severe stages of NAFLD. It could be hypothesised that participants recruited to this trial, were more health conscious and more likely want to take part in a diet related trial after being informed of the unavailability of safe, effective pharmacotherapy for the treatment of NAFLD. Additionally, participants who may have attended standard clinical care for diabetes may have been exposed to general healthy food intake practices by previously advising dietitians or advice provided by attending health practitioner(s). This previous dietary knowledge or existing healthy eating strategies may have influenced some participants in having a better baseline diet than others. Future studies which aim to assess the effects of diet intervention on markers of inflammation, hepatic and metabolic outcomes may require participants to complete a dietary screening tool to confirm 'poor' diet quality prior to enrolment. A study investigating a modified MedDiet for the treatment of moderate to severe depression in Australian patients utilised this approach as a method of standardisation of level of diet at baseline.[355]

Results from the dietary intervention demonstrated that adherence to the MedDiet intervention, delivered by an Accredited Practicing Dietitian, was associated with an improvement in the anti-inflammatory marker adiponectin and a reduction in visceral fat without weight loss. This study also confirmed that a low-fat diet can induce clinically relevant weight loss in individuals with NAFLD, and reduce liver enzymes AST, ALT and GGT and improve insulin sensitivity. However,

there was no improvement in inflammatory markers in the LFD. The main dietary changes observed in the MedDiet group was the consumption of significantly more monounsaturated fatty acids (MUFAs) and omega-3 fatty acid eicosapentaenoic acid (EPA) after the intervention, likely in the form of EVOO and fish/seafood as per the MedDiet recommendations and hampers that were provided. Participants in the MedDiet group also significantly reduced dietary intake of saturated fatty acids (SFAs), refined grains and added sugars. The main dietary changes observed in the low-fat diet group was a significant reduction in total fat intake (as a percentage of total energy) and added sugars. Interestingly, regardless of diet group allocation, all participants significantly improved adherence to the MedDiet and overall diet quality at 12-weeks of intervention. Findings indicate that 'healthy' diet prescription and clinician contact regardless of the diet itself may influence improvements in dietary intake habits in this group. When study participants were pooled for analysis the overall study group significantly reduced consumption of SFAs and refined grains, and significantly increased consumption of MUFAs and whole grains. Participants who were adhering more closely to the MedDiet, determined by moderate to high (7.66-14) PREDIMED scores, had significantly lower levels of pro-inflammatory markers, high sensitivity C-reactive protein (hs-CRP), and leptin and were more insulin resistance than participants who had scores reflective of lower adherence. These findings suggest that dietary improvement focused on whole of diet patterns may be more beneficial in improving inflammation and metabolic risk markers than restrictive type diets, such as a hypocaloric diet (recommended to overweight and obese individuals with NAFLD). Dietary patterns which promote healthy diet and lifestyle behaviours, are inclusive of all food groups and improve chronic underlying inflammation and metabolic control without relying on weight-loss may be most beneficial in this patient group, though longer-term studies and larger sample sizes are required to demonstrate such benefits.

Chronic low-grade inflammation occurs as a result of the accumulation of excess adipose tissue and is considered an underlying pathophysiological feature in the development of NAFLD.[530] Of the inflammatory markers assessed, adiponectin significantly improved following the MedDiet intervention only. Unfavourable (reduced) levels of adiponectin are often associated with obesity and metabolic disease, and tend to be altered by changes in visceral adiposity and improvements in the quality of diet. This was evidenced in the results of the present study, as changes in circulating adiponectin occurred in concurrence with significant reductions in visceral fat, in the absence of weight loss. This finding supports the notion that visceral adipose tissue regulates the secretion of adiponectin into the bloodstream, and not subcutaneous adipose tissue. Existing literature is conflicting with regards to the effect of the MedDiet on adiponectin. The ATTICA cohort study reported that adherence to the MedDiet was associated with an increase in adiponectin and decreased TNF-α concentration in healthy Greek adults, after adjusting for age, sex and central adiposity.[520] More recently, the MÉDITA RCT of patients with newly diagnosed T2DM found that a MedDiet increased adiponectin by 43% whereas the low-fat group remained unchanged even though both groups significantly reduced body weight.[699] Conversely, results from a sub-study of

the PREDIMED study reported that a MedDiet with extra virgin olive oil (EVOO), a MedDiet with nuts and a low-fat diet intervention all increased adiponectin levels patients with T2DM.[700] Each intervention results in weight loss <1kg, though weight loss in patients following the MedDiet interventions was considered significant. These findings suggest that the MedDiet may alter body composition and improve inflammatory markers, though additional studies are warranted to support these findings.

Adiponectin levels were not significantly associated with individual nutrients or food groups, therefore the links between diet and mechanism of action cannot be elucidated from this study. The MedDiet group did, however, significantly increase intake of monounsaturated fatty acids (MUFAs), increase intakes of polyunsaturated fatty acids (PUFAs) and decrease intake of SFA in compliance with the MedDiet prescription. Consumption of MUFA-rich foods increased, mainly in the form of extra virgin olive oil. Olive oil is recognised for its cardioprotective health benefits, generally known to improve inflammation, oxidative stress ad endothelial function. The anti-inflammatory mechanism of both MUFAs and PUFAs is thought to begin with the activation of PPARs, which stimulate oxidation of FFAs and decreases inflammation, IR, glucose control and the accumulation of adipose tissue.[541] Although dietary changes were found to be significant, these changes may not have been substantial enough to promote an anti-inflammatory change in biomarkers of inflammation or the time of intervention was not adequate for noticeable inflammatory changes to occur in this population. However, it can be hypothesised that the anti-inflammatory and anti-oxidant properties of a MedDiet mediate glucose and lipid metabolism, altering adipose tissue without causing weight change.[535] In a physically inactive and obese population, this effect of reducing VF and associated levels of adiponectin without inducing weight loss could be a sustainable and effective treatment.

In contrast, the findings from the low-fat diet group of this study appear to be consistent with other low-fat or low-carbohydrate dietary intervention studies in patients with NAFLD, which demonstrate that a reduction in total fat intake leads to weight loss and subsequent reductions in HOMA-IR and liver enzymes. Participants in the LFD group experienced a clinically relevant, albeit not significant, decrease in body weight of -4.0 ± 2.3kg (3.5%), likely to have been driven by the ~1,000kJ energy deficit observed in this study group over the 12-week intervention. Low-fat diets leading to weight loss and reductions in IR and liver outcomes are well documented in the literature, [refs] and are a direct result of a reduction of dietary fat leading to lower concentrations of total and low-density lipoprotein cholesterol, modulating *de novo* lipogenesis and accumulation of liver triacylglycerol. Diet-induced weight loss is currently the mainstay therapeutic treatment for patients with NAFLD, and short-term restrictive studies often correlate the percentage of weight lost with improvements in health outcomes. Diet and lifestyle interventions that focus on weight loss in NAFLD and other chronic disease populations are often unsustainable, leading to unattainable or unmaintainable weight loss outcomes.

Of the 42 participants with NAFLD recruited to this study, 18 individuals were diagnosed with T2DM, the majority of whom (n=11) were randomised to the low-fat diet. These individuals diagnosed with both NAFLD and T2DM displayed a more severe metabolic and liver disease phenotype at baseline which may have affected their response to dietary intervention. To investigate the impact of diabetes on the change in inflammation, metabolic risk markers and anthropometry and body composition before and after dietary intervention, participants with diabetes were grouped and compared with those without diagnosed diabetes. Participants with diabetes adhering to a low-fat diet significantly reduced HOMA-IR from baseline to 12-weeks, while participants with T2DM in the MedDiet group also reduced HOMA-IR, albeit not significantly. A pooled cohort analysis of participants with T2DM showed that regardless of diet group, intervening with a "healthy" dietary intervention improves overall diet quality resulted in improved outcomes for visceral fat, fasting insulin and IR. In the pooled cohort analysis, HOMA-IR score reduced significantly by a two-unit change in patients with T2DM in the absence of any significant weight loss. Improvements in insulin sensitivity between individuals with and without diagnosed T2DM highlight the difference in response to diet between patients with more severe metabolic phenotypes. Insulin resistance is a surrogate measure for the diagnosis of T2DM and progression of NAFLD, and from a pathophysiological standpoint can be implicated in the response to intervention for patients with a diagnosis of both diseases. These individuals may also be more sensitive to dietary change, increasing their scope for improvement in metabolic outcomes than individuals with only NAFLD. Individuals with diabetes and fatty liver present as a more sensitive patient group in general as evidenced in this group, and should be screened and advised to follow lifestyle interventions that focus on improving diet quality instead of calorie or fat-restriction. Taken together, the results from the individual diet groups and the impact of metabolic disease phenotype may be better treated with a combined intervention to improve diet quality and induce moderate and consistent weight loss. Weight loss may be a minor outcome; however, the amelioration of the metabolic perturbations and inflammation may still be somewhat achieved through dietary changes.

In this study group, regardless of diet allocation, diet quality, composition and overall adherence to a MedDiet (as determined by PREDIMED scores) improved for all participants after 12-weeks of intervention. This result demonstrated that the principles of the MedDiet were accepted amongst multicultural Australian participants, which is particularly encouraging considering the limited research into the acceptability of a MedDiet in non-Mediterranean populations. Although feasibility or acceptability were not main outcomes of this trial, this study adds to the existing data and growing body of literature high supporting adherence to the MedDiet in a multicultural, chronic-diseased cohort in a non-Mediterranean country such as Australia. Studies using similar dietary counselling techniques have also demonstrated high adherence to an *ad libitum* MedDiet intervention in an Australian[275, 355, 701, 702] and Mediterranean[703] setting. While these initial findings are encouraging, there remain potential barriers to successfully translating the MedDiet into the Australian population or the clinical care setting. Macronutrient contributions of a diet are not difficult to

replicate, however the MedDiet is unique in its incorporation of cuisine, key ingredients and cooking methods which allows food and nutrients to have synergistic effect on the body. A key predicted barrier to application of a MD in non-Mediterranean countries, such as Australia, was the adaptability to other cultural preferences.[582] In this clinical trial, methods for translating MedDiet principles to other cultural cuisines was incorporated into the intervention design and delivery.[1] The intervention was delivered by a trained dietitian and involved dietary counselling and goal setting, provision of a food hamper and recipes, a two-week meal plan and other dietary resources. These methods may play an important role in increasing adherence to the MedDiet in this population and should be considered in future clinical studies.

Despite improvements in diet quality and MedDiet adherence, consumption of fruits and vegetables (except for tomatoes which is a key component of the MedDiet), legumes and nuts did not significantly increase in the MedDiet or low-fat diet groups. Non-compliance to these diet recommendations represents an unanticipated barrier to dietary adherence in this group. Indeed, National Health and Nutrition Surveys have highlighted the populations' alarming inadequacy of daily fruit and vegetable intake, with only 5.5% of Australian adults consuming recommended amounts.[582] However, baseline intake of fruit and vegetables in this group did not reflect a standard chronic disease cohort in that nutrient and food group intake did not deviate far above or below RDI's. In contrast, consumption of nuts and legumes was low at baseline and decreased in the pooled cohort from baseline to 12-weeks. Fresh or dried fruit and/or raw unsalted nuts are a rich source of anti-oxidants and lentil/legume-based meals are a rich source of protein in the MedDiet pattern.[1] A more in-depth analysis of barriers to consuming these food groups may be required, as participants may not have been confident in preparing lentil or legume dishes or required more comprehensive, wide-reaching strategies/recipes to include in their daily diet. Dietary consults should ideally be tailored to each individual, their circumstances and role in cooking or food preparation, incorporate practical strategies for selecting and incorporating healthy nutritious foods into the diet, in simple and convenient ways. Addressing the barriers identified in this study will help in achieving desired implementation of the MedDiet in the prevention and management of NAFLD, as well as other chronic diseases.

The Dietary Inflammatory Index (DII) is a novel dietary tool used to assess the inflammatory potential of an individual's diet. DII score was measured at baseline and 12-weeks in this cohort, and the change in DII score was reported for the pooled cohort. At baseline, the DII score of the pooled study group was could be considered 'neutral' and at 12-weeks it was deemed 'anti-inflammatory', although no significant change was seen. At 12-weeks, the DII of this group was similar to the theoretical potential of the prescribed low-fat diet, which although considered 'anti-inflammatory', is not considered to be as anti-inflammatory as the theoretical potential (DII) of the prescribed MedDiet. This study group were consuming an 'anti-inflammatory' diet at 12-weeks, but the score was markedly higher (more 'pro-inflammatory') than the theoretical potential of a

MedDiet. Nevertheless, DII score appeared to be significantly and inversely associated with the PREDIMED score at 12-weeks, which was used a surrogate measure of adherence to the MedDiet. Although adherence to the MedDiet was observed at the end of the intervention period, baseline diets of participants in this group were not extremely poor or representative of a standard chronic disease cohort thus macronutrient and food group changes between baseline and 12-weeks were too modest to identify significant changes in DII score. Whether the DII is only an effective measure of dietary inflammatory potential and dietary change in patients who have a more severe disease phenotype, in comparison to those in the early stages of NAFLD requires further investigation.

This doctoral thesis aimed to explore the added effect of genetic variation and its impact on inflammation and response to diet in this cohort. The prevalence of genetic polymorphisms (or single nucleotide polymorphisms (SNPs)) associated with the production of three cytokines (high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α)) and one adipokine (adiponectin) were examined in chapter 6 of this thesis. This work adds to the paucity of data available regarding the prevalence of risk genotypes of CRP +184, IL-6 -174, TNF-α -308 and adiponectin +276 polymorphisms in a NAFLD population, as well as the involvement of genes in regulating inflammation in NAFLD. In this group, 91% of participants with NAFLD had the risk allele (C) for gene variant CRP +1846 90% of participants had the risk allele (G) for adiponectin +276. For SNP IL-6 -174, although the majority did not carry the risk allele C, many participants (57%) were GG homozygotes which is classified as the high producer phenotype for IL-6. Conversely, only 23% of participants carried the risk allele for the TNF-α -308 polymorphism. A regression analysis found that the presence of SNP TNF-α -308 and CRP +184 predicted levels of their respective serum inflammatory markers following dietary intervention. Irrespective of diet intervention, IL-6 -174 and adiponectin +276 polymorphisms were associated with risk of developing the MetS and T2DM, respectively. These findings suggest that SNPs are implicated in the production of inflammatory markers and likelihood of developing a chronic disease. The meaning of preliminary findings of intervention studies, such as the present study, provide possible implications in the prevention of hepato-metabolic disease. Preventative strategies including genotyping prior to diet or lifestyle intervention may provide a highly reproducible, cost-effective way to allocate patients to the treatment that may be most effective for them. In order for nutrigenomics and nutrigenetics to be rigorously applied to the prevention and treatment of NAFLD, unbiased and consistent results must be generated to serve as the basis for future treatments.[704] While it is clear that both nutrients and genetics are key determinants of health outcomes, the mechanisms which initiate the postprandial responses in inflammation and complex interactions between genes, nutrition's and downstream networks requires further elucidation.[705]

Findings from this chapter also provided a first glance at gene-nutrient interactions in a NAFLD cohort who underwent dietary intervention and experienced an improvement in diet quality. Specifically, the risk genotypes of the TNF-α -308 polymorphism were associated with lower levels

of TNF-α in response to energy (kJ), protein (%E), carbohydrate (%E) and sugar (%E) intake levels above the median intake for the population. Additionally, gene-nutrient interactions were found between risk genotypes of the TNF-α -308 polymorphism and MUFA (%E) intake above the median associated with reductions in circulating TNF-α. Moreover, total fat intake (%E) was associated with circulating TNF-α to the effect of a reduction in the cytokine below median intakes and increases in the cytokine above median intake. TNF-α plays in regulating not only the inflammatory response, but also in regulating lipid metabolism pathways in different cells, tissues and organs. Several studies of gene expression *in vitro*, animal and human models have investigated the effect of dietary fat on TNF-α concentrations.[682, 683] This notion of variant allele responsiveness to change in dietary intake has been discussed extensively in the nutrigenomics literature. To the best of our knowledge, the present study is the first to identify the contributory effects of the TNF-α -308 gene variant and its interaction with dietary fat intake to modulate serum TNF-α levels in a NAFLD population. It is possible that the underlying inflammatory status of participants, potentially due to a pre-existing condition such as NAFLD, and exacerbated by adipose tissue expansion due to increase in fatty acid flux, could determine the extent of the inflammatory response to different dietary fatty acids. In addition to the independent influence of dietary fatty acids on TNFα production, variation in the TNF gene may also contribute to the individual variability observed in TNFα production and TNFA gene expression.[689, 690] The dietary management of individuals with the SNP and genotype in which these different dietary responses occur could allow for more precise and pre-emptive disease treatment. Future research must continue to apply studies of classic nutrigenetics (gene-diet interactions), human and molecular nutrition studies, *in vitro* and *in vivo* models, and extensive and unbiased data from large-scale studies which use high-throughput "omics" techniques to study the effects of nutrients on the body.[706] Omics techniques measure the influence of the diet and the genome on a variety of internal intermediates, including Ribonucleic Acid (RNA) expression; (transcriptomics), epigenetic modifications (epigenetics), metabolites (metabolomics), lipids (lipidomics), proteins (proteomics), and microbial communities (microbiomics).[705] The integration of omics techniques into nutrigenomic studies further understanding of the complexities of nutrient and whole of diet interplay with basic metabolic, inflammatory and other internal processes. This will in turn have important implications for the interpretation of disease processes and scope to identify genetic predictors of disease-relevant responses to diet, and its applicability in the context of personalised nutrition have popular appeal.[705]

Deep phenotyping of liver disease patients will advance our understanding of genetic susceptibility to liver diseases.[707] Deep phenotyping involves the assessment of (a) genomic data, (b) hepatic, metabolic and inflammatory outcomes, (c) anthropometry and body composition, (d) microbiome data, and (e) nutritional information, from well-characterised cohorts such as this study. By using genomic analyses in this way, investigators will be able to assess and stratify which groups or subgroups of patients would respond best to anti-inflammatory (or other) dietary treatments. Such methodologies are needed to progress this field from its early stages to a full nutrigenomic approach

to the prevention and management of NAFLD. Although this study was predominantly exploratory and consisted of a limited number of participants, these findings bring to light potential screening and treatment methods for a significant and prevalent chronic health condition that currently lacks effective treatment methods. Findings are translatable and may be implemented in a clinical care setting, with intensive one-on-one practitioner care. The integration of findings from the present study with existing data may be used to strengthen understanding of gene-mediated inflammation in NAFLD, develop NAFLD-risk scores based on the genetic profile of an individual and provide personalised genotype-based nutrition advice to individuals at high-risk of NAFLD who are more likely to respond. One could hypothesise that using genetics-based personalised or precision nutrition would increase the efficacy of dietary intervention, as the advice provided would be tailored to the individual's response to nutrients at the molecular level which may be associated with optimal health benefits. Such tailored strategies may aid the prevention or management of chronic diet-related disease, such as NAFLD. At present, dietary recommendations based on genotype profiles for NAFLD and indeed other chronic disease states is largely in the experimental phase and although large epidemiological studies have assessed the link between diet, risk factors and genotype, intervention studies are required to verify findings.

7.2 Strengths and Limitations

This study assessed two dietary interventions in patients with NAFLD by utilising a RCT study design which according to National Health and Medical Research Council (NHMRC) is the best source of evidence for effects of interventions.[708] The rigorous, evidence-based published design of the MedDiet intervention established to suit free living, multicultural Australian patients with NAFLD while still encompassing main protective components of a traditional MedDiet was a strength of this intervention study. Both MedDiet and low-fat diet interventions were delivered by accredited practicing dietitians (APDs) who were dedicated to providing a consistent, high-standard of care and support. Participants in both diet groups attended a matched number of appointments and contact/follow-up time with the APD.

Inflammatory markers, the primary outcomes of this doctorate were analysed using reliable and validated methodology and multiplex technology. Multiplex technology has distinct advantages for use in clinical study specimens and is a valid alternative method to ELISA for the evaluation of the majority of cytokines.[709] The range of six cytokine and adipokine markers analysed was also a strength of this study, many dietary intervention trials in this area only include one or two markers hence this study provided a comprehensive measure of the inflammatory profile of the study cohort. Studies which have investigated inflammation in NAFLD have traditionally measured CRP, although its role in the development and progression of NAFLD remains unclear. The cytokine and adipokine markers analysed in the present study were carefully selected to provide a holistic

understanding of the physiological processes involved in NAFLD, via hepatic expression of cytokines and adipose tissue driven synthesis of abnormal cytokine and adipokine production. Another strength of the present study was the examination of four single nucleotide polymorphism from four genes, each of which had data for the corresponding inflammatory markers. The inclusion of gold-standard imaging technique (^{1}H-MRS) to quantify intrahepatic lipid content to estimate hepatic fibrosis and risk of cirrhosis[709, 710] and the inclusion of Transient Elastography, Fibroscan™ to quantify fibrosis were strengths of this study. These measurement techniques of inflammatory markers and hepatic outcomes are robust and were completed by an independent investigator who was blinded to participant study group allocation and time points when scans were taken. Insulin resistance was measured using calculation of HOMA-IR, which is a commonly used, valid and reliable measure of IR in this patient group.[711, 712] Various biomarkers, anthropometric and body composition measured were collected in duplicate by trained researchers in this study population which allowed for a holistic and thorough evaluation of baseline profile and changes over the intervention period.

Dietary data was collected using 3-day food diaries and adherence to specific components of the MedDiet were recorded using the validated PREDIMED checklist at baseline (0-week), 6- and 12-week timepoints. Food diaries are considered to be a robust tool for assessing dietary intake and each food diary was checked by the attending APD while the participant was present in order to confirm data provided and correct any errors. This method ensured that diet data provided was accurate and participants had understood the requirements of keeping a food diary. Similarly, PREDIMED checklists were checked by the APD at each appointment. Although these methods were rigorous, a number of forms of bias can occur in dietary assessment of a study group. The nature of self-reported dietary intake presents a number of barriers including over and underreporting, which is well documented as a limitation in the literature.[713, 714] Biomarkers of dietary adherence such as whole blood fatty acids or excreted metabolites of key dietary components such as hydroxytyrosol as a marker of olive oil consumption may be used in order to overcome this limitation. In future studies, such biomarkers may be implemented to confirm dietary adherence. Another strength of this study was the high completion (93%) rate, indicating that the intervention diet was feasible in this patient group who were attending outpatient liver clinics.

Limitations of this study include a small sample size of forty-two participants, which did not meet the statistically powered sample size of 150-300 required to observe a change in primary outcomes; hs-CRP, IL-6, TNF-α, adiponectin, leptin and resistin. The non-significant changes in inflammatory markers may also be related to this cohort having generally low levels of inflammation at baseline, in the context of other chronic diseased populations. Despite researcher and clinician recruitment efforts, recruitment was slower than expected, and with PhD timeline constraints the doctoral candidate was unable to recruit a larger sample. Although stratified randomisation was used, there was an uneven distribution of participants with diabetes allocated to dietary intervention groups

which may have led to a biased spread of metabolic characteristics, leading to differing scopes for improvement in primary and secondary outcomes between groups. In future dietary intervention studies, both block and stratified randomisation may be an alternative to prevent participant imbalances from occurring. The intervention period for this study was 12-weeks which although adequate, may have provided more insightful results if the intervention period were longer. An intervention period of 6-months could have shown if improvements in inflammation or hepatic outcomes and diet quality were sustained, moreover a follow up period of 12 – 18 months may provide data on sustainability of diet post-intervention. Data collected as part of the MEDINA Study included 6- and 12-month time points which have not yet been analysed, therefore it will be important to assess the long-term inflammatory and metabolic benefits provided by each dietary intervention.

In order to reduce bias associated with clinician contact, both diet intervention groups were provided with an equal number and duration of face-to-face and phone call follow-up appointments during the study intervention period. While this approach ensured equal treatment of all participants, it also provided the 'control' group with dietary support and time with a clinical dietitian that would not have usually been given to participants in a control or standard clinical care group. Matching the number of appointments and providing equal follow up to the control group was not a *true* measure of a control or standard care group as patients in a real-world clinical setting are often lost-to-follow up, do not attend appointments or are not immediately referred to a dietitian. The frequency of appointments would also differ in the real world, outpatient clinical dietitian consultations would occur on average every 3-6 months (less than the administered amount in this study) depending on the severity of disease. Participants in this study attending three face-to-face consultations in 12-weeks which included extensive dietary consultations, as well as three phone-call follow ups at 2-, 4- and 9-weeks. Considering this, and the small sample size, it was difficult to observe a statistically significant change *between* groups within 12-weeks.

Weight loss was not an intended outcome of this trial and recommendations to increase physical activity were not given to participants unless it was a personal goal of the individual. Even so, the low-fat diet group experienced a clinically significant reduction in body weight which may have been influenced by increased physical activity levels. Physical activity is a known influence in the effectiveness of a dietary or lifestyle intervention, hence it should be controlled for as a possible confounder. Physical activity was monitored using a self-reported, validated questionnaire but it was not analysed and reported in this doctoral thesis. Accuracy of the self-reported questionnaire is also questionable as it is subject to under or over reporting. More robust and objective measures of physical activity such as pedometers of accelerometers are warranted to accurately assess the impact of physical activity on results.

A limitation of the use of the DII score in this cohort was the non-measurement of all 45 food items required to calculate the score. Although other studies have also used between 28-35 parameters

instead of the full amount, this study may have required all food parameters to truly measure the anti-inflammatory score of the MedDiet pattern. The anti-inflammatory potential of changes to dietary intake and diet quality were limited by excluded parameters in this study and the effects of the excluded parameters have been discussed in detail in chapter 5. A limitation of chapter 6 (which focused on SNPs in NAFLD) was the absence of healthy control participants genotypes for the same SNPs which would have allowed for comparisons of phenotypic traits of disease. Without a healthy control group, the prevalence of SNPs and associations with disease markers could not be compared to individuals living without NAFLD.

7.3 Future Recommendations and Implications

This research provides evidence that anti-inflammatory diets, such as the MedDiet, may be efficacious and feasible to prescribe to patients with NAFLD in a clinical care setting. The MedDiet may produce anti-inflammatory effects and alter body composition in the absence of body weight loss, as evidenced in the MedDiet arm of the present study. Clinical trials with larger sample sizes and longer intervention and follow-up periods are required to confirm these findings. The optimal type of dietary pattern for the management of NAFLD requires further investigation especially in multiethnic, western populations.

This research strengthens existing literature for weight loss and reduction in metabolic and liver outcomes following the low-fat diet, which currently prescribed as standard care for patients who present to outpatient clinics for NAFLD. The metabolic and hepatic benefits that occur due to weight loss cannot be overlooked, however sustainability of the changes in body weight should be targeted. A dietary intervention which encompasses the health benefits of an anti-inflammatory diet whilst also inducing weight loss, such as a hypocaloric MedDiet or an isocaloric MedDiet alongside recommendations of moderate levels of physical activity, should be considered. Studies should also focus on long-term adherence and follow-up to investigate sustainability of diet, weight loss and associated clinical outcomes (inflammatory, metabolic, liver, anthropometry and body composition), rather than intense interventions, in this group of participants.

Adherence to the MedDiet, as determined by PREDIMED scores, were used as a surrogate marker of diet quality and indicated that both intervention groups improved nutritional adequacy of their diet. This finding is indicative that healthy diet interventions are feasible in the clinical health care setting and will likely be correlated with improvements in inflammatory and metabolic status of NAFLD patients. Prevalence rates of NAFLD are rising in parallel with rates of obesity and T2DM, therefore it is particularly important that this silent, asymptomatic disease be managed prior to a dramatic increase in healthcare burden and economic costs. Progression of NAFLD to irreversible disease states, liver and CVD-related mortality are costly and require a multidisciplinary approach

for optimal management. Effective strategies that consider long-term improvements, participant preference and quality of life are needed to treat patients who present with early stages of NAFLD.

Recruitment of patients with less progressed NAFLD was difficult during the recruitment phase of this study. This was due to the low prevalence of NAFLD patients in the clinical setting, most of whom presented with high-risk stages of NAFLD and NASH.[47] Future studies should aim to recruit from a wider range of settings such as from general practitioner or medical clinics, obesity and diabetes hospital outpatient clinics, and fatty liver or liver hospital outpatient clinics. Increasing literature has indicated that patients who have undiagnosed NAFLD are not being screened whilst attending obesity or diabetes clinics, and are consequently not treated for the disease that will silently progress. Patients with NAFLD tend to be motivated as there are no drug trials available to them and referring heptologists are supportive of their involvement in clinical dietary intervention studies.

Emerging clinical studies and therapies for treatment of patients with NAFLD are now targeting gut microbiota, as accumulating evidence implicates the gut in the development and progression of NAFLD.[715] Indeed, the multiple hits hypothesis for the pathogenesis of NAFLD included the gut microbiome as a potential underlying mechanism of liver "insult", likely contribute to overall NAFLD pathophysiology.[125] Moreover, bacterial translocation and the entry of bacteria-derived products into the portal circulation may induce proinflammatory cascades in the liver (production of IL-6 and TNF-α).[715] Few human studies have assessed the link between gut dysbiosis to the severity of hepatic inflammation and/or fibrosis, however those that have concluded that there may be a future role for the gut microbiome to be used as a non-invasive tool to measure diagnosis, disease severity and monitoring for patients with NASH.[716, 717] Potential therapies that alter gut microbiota through treatment, such as the inclusion of prebiotics, probiotics, synbiotics, or antibiotics represent an evolving area of research in NAFLD.[715] Therapeutic options which target multiple pathophysiologic pathways may be beneficial for the treatment of patients with NAFLD and require further testing in this patient population.

7.4 Conclusion

This doctoral study provides emerging evidence of the beneficial anti-inflammatory effects of a Mediterranean diet intervention in patients with NAFLD, in the absence of weight loss. The Mediterranean diet was more effective in improving adiponectin and visceral fat, without reducing body weight. Whereas the low-fat diet was superior in reducing insulin resistance and liver enzymes, with weight loss experienced by those who followed this diet. When the cohort was pooled for analysis, it was found that improvements in diet quality occurred in all study participants indicating that 'healthy' dietary intervention has the potential to be an effective clinical intervention and should therefore be promoted in the management of NAFLD. Moreover, this research highlights the potential of nutrigenetic and nutrigenomic approaches to improve our understanding of the complex interactions occurring between the human genome, nutrients and inflammatory perturbations, and how inclusion of genomic techniques may assist dietary strategies in the prevention and treatment of patients with NAFLD.

This research demonstrates that an *ad libitum* Mediterranean diet can be successfully adhered to in a multiethnic Australian cohort of patients with NAFLD. Targeted strategies to incorporate particular food groups; vegetables, fruits, legumes, nuts, with dietary counselling methods that suit individual participants during an intervention period may further improve adherence to diet and is likely to achieve desired benefits with regards to reduced inflammation. High risk populations, including those who have metabolic conditions including T2DM or the MetS, are likely to benefit from a more efficient screening and diagnosis process, proactive treatment strategy and regular follow-up. Therapeutic strategies targeted at ameliorating risk factors such as elevated inflammatory markers and insulin resistance will be beneficial in reducing or slowing progression of NAFLD, as well as a range of other health benefits considering the strong overlap between NAFLD and other highly prevalent medical conditions such as obesity, T2DM and CVD.

Future studies are warranted to assess the impact of modified dietary treatments, such as a hypocaloric Mediterranean type-diet or an isocaloric Mediterranean type-diet with moderate physical activity, on inflammation, metabolic control and adiposity over longer periods of time. Importance should be placed on sustainability of adherence to diet and changes in weight, adiposity, inflammation and other clinical biomarkers. Moreover, evidence is accumulating for the incorporation of nutri-genomics/genetics and gut microbiota measurements in this population, which warrants the inclusion of such techniques into high quality clinical trials to evaluate the impact of diet and lifestyle modification in patients with NAFLD.

References

1. George E, Kucianski T, Mayr H, Moschonis G, Tierney A, Itsiopoulos C. A Mediterranean diet model in Australia: strategies for translating the traditional Mediterranean diet into a multicultural setting. Nutrients. 2018;10(4):465.
2. Xavier A, Zacconi F, Gainza C, Cabrera D, Arrese M, Uribe S, et al. Intrahepatic fatty acids composition as a biomarker of NAFLD progression from steatosis to NASH by using 1 H-MRS. RSC Advances. 2019;9(72):42132-9.
3. Dongiovanni P, Valenti L. A Nutrigenomic Approach to Non-Alcoholic Fatty Liver Disease. International journal of molecular sciences. 2017;18(7):1534.
4. Eslamparast T, Tandon P, Raman M. Dietary Composition Independent of Weight Loss in the Management of Non-Alcoholic Fatty Liver Disease. Nutrients. 2017;9(8):800.
5. Angulo P. Nonalcoholic fatty liver disease. J New England Journal of Medicine. 2002;346(16):1221-31.
6. Pierantonelli I, Svegliati-Baroni GJT. Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH. 2019;103(1):e1-e13.
7. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999;30(6):1356-62.
8. Diehl A, Goodman Z, Ishak K. Alcohollike liver disease in nonalcoholics: a clinical and histologic comparison with alcohol-induced liver injury. Gastroenterology. 1988;95(4):1056-62.
9. Sanyal AJ. Past, present and future perspectives in nonalcoholic fatty liver disease. Nature Reviews Gastroenterology Hepatology. 2019:1.
10. Obika M, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. Experimental diabetes research. 2011;2012.
11. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. 2005;288(2):E462-E8.
12. Lee SS, Park SHJWjogW. Radiologic evaluation of nonalcoholic fatty liver disease. 2014;20(23):7392.
13. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology. 2010;51(2):679-89.
14. Radziuk J, Pye S. Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. Diabetes/metabolism research and reviews. 2001;17(4):250-72.
15. Loria P, Lonardo A, Anania F. Liver and diabetes. A vicious circle. Hepatology Research. 2013;43(1):51-64.
16. Levinthal GN, Tavill AS. Liver disease and diabetes mellitus. Clin Diabetes. 1999;17(2):73-93.
17. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-48.
18. Chatrath H, Vuppalanchi R, Chalasani N, editors. Dyslipidemia in patients with nonalcoholic fatty liver disease. Seminars in liver disease; 2012: Thieme Medical Publishers.

19. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, et al. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. The Journal of clinical investigation. 2009;119(2):315-22.

20. Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. Comprehensive Physiology. 2011;8(1):1-22.

21. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. Cell metabolism. 2008;7(2):95-6.

22. Hatting M, Tavares CD, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. Annals of the New York Academy of Sciences. 2018;1411(1):21.

23. Marjot T, Sbardella E, Moolla A, Hazlehurst J, Tan G, Ainsworth M, et al. Prevalence and severity of non-alcoholic fatty liver disease are underestimated in clinical practice: impact of a dedicated screening approach at a large university teaching hospital. Diabetic Medicine. 2018;35(1):89-98.

24. Cotter TG, Rinella M. NAFLD 2020: The State of the Disease. Gastroenterology. 2020.

25. Dongiovanni P, Paolini E, Corsini A, Sirtori CR, Ruscica M. Nonalcoholic fatty liver disease or metabolic dysfunction-associated fatty liver disease diagnoses and cardiovascular diseases: From epidemiology to drug approaches. Eur J Clin Invest. 2021:e13519-e.

26. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.

27. Loomba R, Sanyal AJ. The global NAFLD epidemic. Nature Reviews Gastroenterology and Hepatology. 2013;10(11):686.

28. Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology. 2016;64(5):1577-86.

29. Economics DA. The economic cost and health burden of liver diseases in Australia. Kingston: The Gastroenterological Society of Australia/Australian Liver Association. 2013.

30. Adams LA, Roberts SK, Strasser SI, Mahady SE, Powell E, Estes C, et al. Nonalcoholic fatty liver disease burden: Australia, 2019–2030. Journal of Gastroenterology and Hepatology. 2020.

31. Maier S, Wieland A, Cree-Green M, Nadeau K, Sullivan S, Lanaspa MA, et al. Lean NAFLD: An underrecognized and challenging disorder in medicine. Reviews in Endocrine and Metabolic Disorders. 2021:1-16.

32. Lu FB, Zheng KI, Rios RS, Targher G, Byrne CD, Zheng MH. Global epidemiology of lean non-alcoholic fatty liver disease: A systematic review and meta-analysis. Journal of Gastroenterology and Hepatology. 2020;35(12):2041-50.

33. Fan J-G, Kim S-U, Wong VW-S. New trends on obesity and NAFLD in Asia. Journal of hepatology. 2017;67(4):862-73.

34. Marchesini G, Mazzotti A. NAFLD incidence and remission: Only a matter of weight gain and weight loss? Journal of hepatology. 2015;62(1):15-7.

35. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-57.

36. Whalley S, Puvanachandra P, Desai A, Kennedy H. Hepatology outpatient service provision in secondary care: a study of liver disease incidence and resource costs. Clinical medicine. 2007;7(2):119-24.

37. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer MJH. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. 2016;64(1):73-84.

38. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. Journal of hepatology. 2017;67(4):829-46.

39. Byrne CD, Targher G. NAFLD: a multisystem disease. Journal of hepatology. 2015;62(1):S47-S64.

40. Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. Annual Review of Pathological Mechanical Disease. 2010;5:145-71.

41. Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. Nature reviews Gastroenterology & hepatology. 2013;10(11):656.

42. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Digestive diseases. 2010;28(1):155-61.

43. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis. 2007;191(2):235-40.

44. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature Reviews Gastroenterology and Hepatology. 2018;15(1):11.

45. Health NIf, Excellence C. Non-alcoholic fatty liver disease (NAFLD): assessment and management. National Institute for Health and Care Excellence London; 2016.

46. Liver EAftSot, Diabetes EAftSo. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Obesity facts. 2016;9(2):65-90.

47. George ES, Roberts SK, Nicoll AJ, Reddy A, Paris T, Itsiopoulos C, et al. Non-alcoholic fatty liver disease patients attending two metropolitan hospitals in Melbourne, Australia: high risk status and low prevalence. Internal medicine journal. 2018;48(11):1369-76.

48. Dunn W, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. The American journal of gastroenterology. 2008;103(9):2263.

49. Bertolotti M, Lonardo A, Mussi C, Baldelli E, Pellegrini E, Ballestri S, et al. Nonalcoholic fatty liver disease and aging: epidemiology to management. World journal of gastroenterology: WJG. 2014;20(39):14185.

50. Adams LA, Lymp JF, Sauver JS, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129(1):113-21.

51. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. Journal of hepatology. 2008;49(4):608-12.

52. Vernon G, Baranova A, Younossi Z. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Alimentary pharmacology & therapeutics. 2011;34(3):274-85.

53. Frith J, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. Gerontology. 2009;55(6):607-13.

54. Kagansky N, Levy S, Keter D, Rimon E, Taiba Z, Fridman Z, et al. Non-alcoholic fatty liver disease–a common and benign finding in octogenarian patients. Liver International. 2004;24(6):588-94.

55. Ascha MS, Hanouneh IA, Lopez R, Tamimi TAR, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology. 2010;51(6):1972-8.

56. Hashimoto E, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K, et al. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Journal of gastroenterology. 2009;44(19):89-95.

57. Liew P-L, Lee W-J, Wang W, Lee Y-C, Chen W-Y, Fang C-L, et al. Fatty liver disease: predictors of nonalcoholic steatohepatitis and gallbladder disease in morbid obesity. Obesity surgery. 2008;18(7):847-53.

58. Chen Z-w, Chen L-y, Dai H-l, Chen J-h, Fang L-z. Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. Journal of Zhejiang University Science B. 2008;9(8):616.

59. Sorrentino P, Tarantino G, Conca P, Perrella A, Terracciano ML, Vecchione R, et al. Silent non-alcoholic fatty liver disease—a clinical–histological study. Journal of hepatology. 2004;41(5):751-7.

60. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. American journal of epidemiology. 2013;178(1):38-45.

61. Kallwitz ER, Kumar M, Aggarwal R, Berger R, Layden-Almer J, Gupta N, et al. Ethnicity and nonalcoholic fatty liver disease in an obesity clinic: the impact of triglycerides. Digestive diseases and sciences. 2008;53(5):1358-63.

62. Papatheodoridis GV, Goulis J, Christodoulou D, Manolakopoulos S, Raptopoulou M, Andrioti E, et al. High prevalence of elevated liver enzymes in blood donors: associations with male gender and central adiposity. European journal of gastroenterology & hepatology. 2007;19(4):281-7.

63. Gutierrez-Grobe Y, Ponciano-Rodríguez G, Ramos MH, Uribe M, Méndez-Sánchez N. Prevalence of non alcoholic fatty liver disease in premenopausal, posmenopausal and polycystic ovary syndrome women. The role of estrogens. Annals of hepatology. 2010;9(4):402-9.

64. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140(1):124-31.

65. Wagenknecht LE, Scherzinger AL, Stamm ER, Hanley AJ, Norris JM, Chen YDI, et al. Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. Obesity. 2009;17(6):1240-6.

66. Rich NE, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, et al. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis. Clinical Gastroenterology and Hepatology. 2018;16(2):198-210. e2.

67. Wei JL, Leung JC-F, Loong TC-W, Wong GL-H, Yeung DK-W, Chan RS-M, et al. Prevalence and severity of nonalcoholic fatty liver disease in non-obese patients: a population study using proton-magnetic resonance spectroscopy. The American journal of gastroenterology. 2015;110(9):1306.

68. Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;136(5):1585-92.

69. Pankow JS, Folsom AR, Cushman M, Borecki IB, Hopkins PN, Eckfeldt JH, et al. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis. 2001;154(3):681-9.

70. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. The American journal of gastroenterology. 2001;96(10):2957-61.

71. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nature genetics. 2008;40(12):1461-5.

72. Del Campo JA, Gallego-Durán R, Gallego P, Grande L. Genetic and epigenetic regulation in nonalcoholic fatty liver disease (NAFLD). International journal of molecular sciences. 2018;19(3):911.

73. Dongiovanni P, M Anstee Q, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. Current pharmaceutical design. 2013;19(29):5219-38.

74. Kalafati I-P, Borsa D, Dedoussis GV. The genetics of nonalcoholic fatty liver disease: role of diet as a modifying factor. Current Nutrition Reports. 2014;3(3):223-32.

75. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet. 2011;7(3):e1001324.

76. Kovac U, Rozman D. Genetics of Non-alcoholic Fatty Liver Disease. eLS. 2015:1-8.

77. de Alwis NMW, Day CP. Genes and nonalcoholic fatty liver disease. Current diabetes reports. 2008;8(2):156.

78. Wang Jk, Feng Zw, Li Yc, Li Qy, Tao Xy. Association of tumor necrosis factor-α gene promoter polymorphism at sites-308 and-238 with non-alcoholic fatty liver disease: A meta-analysis. Journal of gastroenterology and hepatology. 2012;27(4):670-6.

79. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. Digestive Liver Disease. 2015;47(3):181-90.

80. Ortiz-Lopez C, Lomonaco R, Orsak B, Finch J, Chang Z, Kochunov VG, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes care. 2012;35(4):873-8.

81. Fan JG. Impact of non-alcoholic fatty liver disease on accelerated metabolic complications. Journal of digestive diseases. 2008;9(2):63-7.

82. Milić S, Lulić D, Štimac D. Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations. World journal of gastroenterology: WJG. 2014;20(28):9330.

83. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. JAMA. 2003;289(2):187-93.

84. Angulo P. Obesity and nonalcoholic fatty liver disease. Nutrition reviews. 2007;65(suppl_1):S57-S63.

85. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of

hepatic steatosis in the general population. American Journal of Physiology-Endocrinology and Metabolism. 2005;288(2):E462-E8.

86. Marcos A, Fisher RA, Ham JM, Olzinski AT, Shiffman ML, Sanyal AJ, et al. Selection and outcome of living donors for adult to adult right lobe transplantation. Transplantation. 2000;69(11):2410-5.

87. Hilden M, Christoffersen P, Juhl E, Dalgaard J. Liver histology in a 'normal'population—examinations of 503 consecutive fatal traffic casualties. Scandinavian journal of gastroenterology. 1977;12(5):593-7.

88. Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. Human Pathology. 1989;20(6):594-8.

89. Gholam PM, Kotler DP, Flancbaum LJ. Liver pathology in morbidly obese patients undergoing Roux-en-Y gastric bypass surgery. Obesity surgery. 2002;12(1):49-51.

90. Chung GE, Kim D, Kwark MS, Kim W, Yim JY, Kim YJ, et al. Visceral adipose tissue area as an independent risk factor for elevated liver enzyme in nonalcoholic fatty liver disease. 2015;94(9).

91. Lira FS, Rosa JC, dos Santos RV, Venancio DP, Carnier J, de Lima Sanches P, et al. Visceral fat decreased by long-term interdisciplinary lifestyle therapy correlated positively with interleukin-6 and tumor necrosis factor–α and negatively with adiponectin levels in obese adolescents. Metabolism. 2011;60(3):359-65.

92. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30(5):1212-8.

93. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver International. 2009;29(1):113-9.

94. Prashanth M, Ganesh H, Vima M, John M, Bandgar T, Joshi SR, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. J Assoc Physicians India. 2009;57(3):205-10.

95. Kasturiratne A, Weerasinghe S, Dassanayake AS, Rajindrajith S, de Silva AP, Kato N, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. Journal of Gastroenterology and Hepatology. 2013;28(1):142-7.

96. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4):738-44.

97. Davì G, Guagnano MT, Ciabattoni G, Basili S, Falco A, Marinopiccoli M, et al. Platelet activation in obese women: role of inflammation and oxidant stress. JAMA. 2002;288(16):2008-14.

98. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology. 1999;116(6):1413-9.

99. Calonge N, Petitti DB, DeWitt TG, Dietrich AJ, Gordis L, Gregory KD, et al. Screening for type 2 diabetes mellitus in adults: US Preventive Services Task Force recommendation statement. Annals of Internal Medicine. 2008;148(11):846-54.

100. Association AD. Standards of medical care in diabetes - 2008 Diabetes Care, 31 (1, Suppl. 1) S12-S54. 2008.

101. Chew DP, Scott IA, Cullen L, French JK, Briffa TG, Tideman PA, et al. National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand: Australian clinical guidelines for the management of acute coronary syndromes 2016. Medical Journal of Australia. 2016;205(3):128-33.

102. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. The Lancet. 2005;365(9468):1415-28.

103. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation. 2005;112(17):2735-52.

104. Expert Panel on Detection E. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486.

105. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA. 2002;287(3):356-9.

106. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. Current opinion in lipidology. 2005;16(4):421-7.

107. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. The American Journal of Gastroenterology. 2009;104(4):861.

108. Ryoo J-H, Choi J-M, Moon SY, Suh YJ, Shin J-Y, Shin HC, et al. The clinical availability of non alcoholic fatty liver disease as an early predictor of the metabolic syndrome in Korean men: 5-year's prospective cohort study. Atherosclerosis. 2013;227(2):398-403.

109. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, Network NCR. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology. 2011;53(3):810-20.

110. Stepanova M, Rafiq N, Younossi ZM. Components of metabolic syndrome are independent predictors of mortality in patients with chronic liver disease: a population-based study. Gut. 2010;59(10):1410-5.

111. James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. Journal of hepatology. 1998;29(3):495-501.

112. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003;37(6):1286-92.

113. Fleming K, Morton J, Barbatis C, Burns J, Canning S, McGee J. Mallory bodies in alcoholic and non-alcoholic liver disease contain a common antigenic determinant. Gut. 1981;22(5):341-4.

114. Ludwig J, Viggiano TR, Mcgill DB, Oh B, editors. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clinic Proceedings; 1980.

115. Day CP, James OF. Steatohepatitis: a tale of two “hits”? : Elsevier; 1998.

116. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes. 2001;50(8):1844-50.

117. Abenavoli L, Milic N, Di Renzo L, Preveden T, Medić-Stojanoska M, De Lorenzo A. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. World journal of gastroenterology. 2016;22(31):7006.

118. Eslam M, Newsome PN, Anstee QM, Targher G, Gomez MR, Zelber-Sagi S, et al. A new definition for metabolic associated fatty liver disease: an international expert consensus statement. Journal of hepatology. 2020.

119. Eslam M, Sanyal AJ, George J, Sanyal A, Neuschwander-Tetri B, Tiribelli C, et al. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology. 2020;158(7):1999-2014. e1.

120. Tilg H, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. Nature Reviews Gastroenterology & Hepatology. 2020:1-2.

121. Fouad Y, Waked I, Bollipo S, Gomaa A, Ajlouni Y, Attia D. What's in a name? Renaming 'NAFLD'to 'MAFLD'. Liver International. 2020.

122. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. The Journal of clinical investigation. 2005;115(5):1343-51.

123. Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. 2016;31(5):936-44.

124. Chen Z, Yu R, Xiong Y, Du F, Zhu S. A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. Lipids in Health Disease. 2017;16(1):203.

125. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology. 2010;52(5):1836-46.

126. Kirpich IA, Marsano LS, McClain CJ. Gut–liver axis, nutrition, and non-alcoholic fatty liver disease. Clinical biochemistry. 2015;48(13-14):923-30.

127. Chen Z, Yu R, Xiong Y, Du F, Zhu SJLiH, Disease. A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. Lipids in Health. 2017;16(1):203.

128. Day CP. From fat to inflammation. Gastroenterology. 2006;130(1):207-10.

129. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li Z-W, Karin M, et al. Reversal of obesity-and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ. Science. 2001;293(5535):1673-7.

130. Arkan MC, Hevener AL, Greten FR, Maeda S, Li Z-W, Long JM, et al. IKK-β links inflammation to obesity-induced insulin resistance. Nature medicine. 2005;11(2):191.

131. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. Nature medicine. 2005;11(2):183.

132. Samuel VT, Liu Z-X, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. Journal of Biological Chemistry. 2004;279(31):32345-53.

133. Ueki K, Kondo T, Tseng Y-H, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. Proceedings of the national academy of sciences. 2004;101(28):10422-7.

134. Yang R, Wilcox DM, Haasch DL, Jung PM, Nguyen PT, Voorbach MJ, et al. Liver-specific knockdown of JNK1 up-regulates proliferator-activated receptor γ coactivator 1β and increases plasma triglyceride despite reduced glucose and insulin levels in diet-induced obese mice. Journal of Biological Chemistry. 2007;282(31):22765-74.

135. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7.

136. Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends in Endocrinology & Metabolism. 2008;19(10):371-9.

137. Saqib U, Sarkar S, Suk K, Mohammad O, Baig MS, Savai R. Phytochemicals as modulators of M1-M2 macrophages in inflammation. Oncotarget. 2018;9(25):17937.

138. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259(5091):87-91.

139. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature. 1997;389(6651):610-4.

140. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-α in sera of obese patients: fall with weight loss. The Journal of Clinical Endocrinology & Metabolism. 1998;83(8):2907-10.

141. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. The Journal of clinical investigation. 1995;95(5):2111-9.

142. Crespo J, Cayón A, Fernández-Gil P, Hernández-Guerra M, Mayorga M, Domínguez-Díez A, et al. Gene expression of tumor necrosis factor α and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. Hepatology. 2001;34(6):1158-63.

143. Lesmana CRA, Hasan I, Budihusodo U, Gani RA, Krisnuhoni E, Akbar N, et al. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. Journal of digestive diseases. 2009;10(3):201-6.

144. Barbuio R, Milanski M, Bertolo MB, Saad MJ, Velloso LcA. Infliximab reverses steatosis and improves insulin signal transduction in liver of rats fed a high-fat diet. Journal of Endocrinology. 2007;194(3):539-50.

145. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti–TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes. 1996;45(7):881-5.

146. Bernstein LE, Berry J, Kim S, Canavan B, Grinspoon SK. Effects of etanercept in patients with the metabolic syndrome. Archives of internal medicine. 2006;166(8):902-8.

147. Braunersreuther V, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. World journal of gastroenterology: WJG. 2012;18(8):727.

148. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science. 1996;274(5291):1379-83.

149. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. American Journal of Gastroenterology. 2008;103(6):1372-9.

150. Beuchler C, Bauer S. IL-6 in non-alcoholic fatty liver disease-good, evil or both. Endocrinology and Metabolic Syndrome. 2011;1:1-3.

151. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. The Journal of clinical investigation. 1998;102(7):1369-76.

152. Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. Endocrinology. 2005;146(8):3417-27.

153. Kopp H-P, Kopp C, Festa A, Krzyzanowska K, Kriwanek S, Minar E, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23(6):1042-7.

154. Rabelo F, Oliveira CP, Faintuch J, Mazo DF, Lima VM, Stefano JT, et al. Pro-and anti-inflammatory cytokines in steatosis and steatohepatitis. Obesity surgery. 2010;20(7):906-12.

155. Zimmermann E, Anty R, Tordjman J, Verrijken A, Gual P, Tran A, et al. C-reactive protein levels in relation to various features of non-alcoholic fatty liver disease among obese patients. Journal of hepatology. 2011;55(3):660-5.

156. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon III RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. circulation. 2003;107(3):499-511.

157. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. New England journal of medicine. 2000;342(12):836-43.

158. Targher G. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. Journal of hepatology. 2006;45(6):879-81.

159. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. Diabetic Medicine. 2005;22(10):1354-8.

160. Hui JM, Farrell GC, Kench JG, George J. High sensitivity C-reactive protein values do not reliably predict the severity of histological changes in NAFLD. Hepatology. 2004;39(5):1458-9.

161. Chiang C-H, Huang C-C, Chan W-L, Chen J-W, Leu H-B. The severity of non-alcoholic fatty liver disease correlates with high sensitivity C-reactive protein value and is independently associated with increased cardiovascular risk in healthy population. Clinical biochemistry. 2010;43(18):1399-404.

162. Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. Journal of gastroenterology. 2007;42(7):573-82.

163. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature Reviews Immunology. 2006;6(10):772-83.

164. Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. International journal of molecular sciences. 2017;18(6):1321.

165. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature medicine. 2002;8(7):731-7.

166. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, et al. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochemical and biophysical research communications. 2003;301(4):1045-50.

167. Neuman MG, Cohen LB, Nanau RM. Biomarkers in nonalcoholic fatty liver disease. Canadian Journal of Gastroenterology and Hepatology. 2014;28.

168. Finelli C, Tarantino G. What is the role of adiponectin in obesity related non-alcoholic fatty liver disease? World journal of gastroenterology: WJG. 2013;19(6):802.

169. Shimada M, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, et al. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. American Journal of Gastroenterology. 2007;102(9):1931-8.

170. Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, et al. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic Beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. American Journal of Gastroenterology. 2005;100(11):2438-46.

171. Machado MV, Coutinho J, Carepa F, Costa A, Proença H, Cortez-Pinto H. How adiponectin, leptin, and ghrelin orchestrate together and correlate with the severity of nonalcoholic fatty liver disease. European journal of gastroenterology & hepatology. 2012;24(10):1166-72.

172. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. The journal of clinical endocrinology & metabolism. 2005;90(6):3498-504.

173. Jarrar M, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Alimentary pharmacology & therapeutics. 2008;27(5):412-21.

174. Aller R, de Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, et al. Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. Digestive diseases and sciences. 2008;53(4):1088-92.

175. Wong VWS, Hui AY, Tsang SWC, Chan JLY, Tse AML, Chan KF, et al. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. Clinical Gastroenterology and Hepatology. 2006;4(9):1154-61.

176. Angulo P, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. Journal of hepatology. 2004;41(6):943-9.

177. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Liddle C, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology. 2002;36(2):403-9.

178. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine–endothelial cell interactions. Biochemical and biophysical research communications. 2004;314(2):415-9.

179. Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, et al. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to

insulin resistance. The journal of clinical endocrinology & metabolism. 2006;91(3):1081-6.

180. Aller R, De Luis D, Izaola O, Sagrado MG, Conde R, Velasco M, et al. Influence of visfatin on histopathological changes of non-alcoholic fatty liver disease. Digestive diseases and sciences. 2009;54(8):1772-7.

181. Haukeland JW, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, et al. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. Journal of hepatology. 2006;44(6):1167-74.

182. Jung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences. 2014;15(4):6184-223.

183. Daly AK, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. Expert review of gastroenterology & hepatology. 2011;5(2):253-63.

184. Oliveira CP, Stefano JT. Genetic polymorphisms and oxidative stress in non-alcoholic steatohepatitis (NASH): a mini review. Clinics and research in hepatology and gastroenterology. 2015;39:S35-S40.

185. Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: Interleukin-6− 174G/C polymorphism is associated with non-alcoholic steatohepatitis. Digestive and Liver Disease. 2009;41(11):823-8.

186. Chang YH, Huang CN, Shiau MY. The C-174G promoter polymorphism of the interleukin-6 (IL-6) gene that affects insulin sensitivity in Caucasians is not involved in the pathogenesis of Taiwanese type 2 diabetes mellitus. European cytokine network. 2004;15(2):117-9.

187. Giannitrapani L, Soresi M, Balasus D, Licata A, Montalto G. Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. World journal of gastroenterology: WJG. 2013;19(16):2449.

188. Miller AM, Wang H, Bertola A, Park O, Horiguchi N, Hwan Ki S, et al. Inflammation-associated interleukin-6/signal transducer and activator of transcription 3 activation ameliorates alcoholic and nonalcoholic fatty liver diseases in interleukin-10–deficient mice. Hepatology. 2011;54(3):846-56.

189. Li X-L, Sui J-Q, Lu L-L, Zhang N-N, Xu X, Dong Q-Y, et al. Gene polymorphisms associated with non-alcoholic fatty liver disease and coronary artery disease: a concise review. Lipids in health and disease. 2016;15(1):1-8.

190. Zhang H, Mo X, Hao Y, Gu D. Association between polymorphisms in the adiponectin gene and cardiovascular disease: a meta-analysis. BMC medical genetics. 2012;13(1):40.

191. Gupta AC, Misra R, Sakhuja P, Singh Y, Basir SF, Sarin SK. Association of adiponectin gene functional polymorphisms (− 11377C/G and+ 45T/G) with nonalcoholic fatty liver disease. Gene. 2012;496(1):63-7.

192. Liu J, Xing J, Wang B, Wei C, Yang R, Zhu Y, et al. Correlation between adiponectin gene rs1501299 polymorphism and nonalcoholic fatty liver disease susceptibility: a systematic review and meta-analysis. Medical science monitor: international medical journal of experimental and clinical research. 2019;25:1078.

193. Shin M-J, Jang Y, Koh SJ, Chae JS, Kim OY, Lee JE, et al. The association of SNP276G> T at adiponectin gene with circulating adiponectin and insulin resistance in response to mild weight loss. International journal of obesity. 2006;30(12):1702-8.

194. Lee J, Yoon K, Ryu S, Chang Y, Kim H-R. High-normal levels of hs-CRP predict the development of non-alcoholic fatty liver in healthy men. PLoS One. 2017;12(2):e0172666.

195. Eiriksdottir G, Smith A, Aspelund T, Hafsteinsdottir S, Olafsdottir E, Launer L, et al. The interaction of adiposity with the CRP gene affects CRP levels: age, gene/environment susceptibilty-Reykjavik study. International journal of obesity. 2009;33(2):267-72.

196. Saadeh S. Nonalcoholic fatty liver disease and obesity. Nutrition in Clinical Practice. 2007;22(1):1-10.

197. Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1 H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. European radiology. 2011;21(1):87-97.

198. McPherson S, Jonsson JR, Cowin GJ, O'Rourke P, Clouston AD, Volp A, et al. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. Journal of hepatology. 2009;51(2):389-97.

199. Hübscher S. Histological assessment of non-alcoholic fatty liver disease. Histopathology. 2006;49(5):450-65.

200. Foucher J, Chanteloup E, Vergniol J, Castera L, Le Bail B, Adhoute X, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut. 2006;55(3):403-8.

201. Friedrich–Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology. 2008;134(4):960-74. e8.

202. Kemp W, Roberts S. FibroScan and transient elastography. Australian family physician. 2013;42(7):468.

203. Abenavoli L, Beaugrand M. Transient elastography in non-alcoholic fatty liver disease. Annals of hepatology. 2012;11(2):172-8.

204. Day CP. Non-alcoholic fatty liver disease: current concepts and management strategies. Clinical Medicine. 2006;6(1):19.

205. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. The American journal of gastroenterology. 1999;94(9):2467-74.

206. Day C. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? Gut. 2002;50(5):585-8.

207. Day C, Saksena S. Non-alcoholic steatohepatitis: definitions and pathogenesis. Journal of gastroenterology and hepatology. 2002;17:S377-S84.

208. Brunt EM. Nonalcoholic steatohepatitis (NASH): further expansion of this clinical entity? Liver. 1999;19(4):263-4.

209. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-21.

210. Kleiner DE, Makhlouf HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. Clinics in liver disease. 2016;20(2):293-312.

211. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004;40(6):1387-95.

212. Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. Gastroenterology. 2008;134(6):1682-98.

213. Tahan V, Canbakan B, Balci H, Dane F, Akin H, Can G, et al. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. Hepato-gastroenterology. 2008;55(85):1433-8.

214. Pantsari MW, Harrison SA. Nonalcoholic fatty liver disease presenting with an isolated elevated alkaline phosphatase. Journal of clinical gastroenterology. 2006;40(7):633-5.

215. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC gastroenterology. 2006;6(1):33.

216. Gastaldelli A, Kozakova M, Højlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. Hepatology. 2009;49(5):1537-44.

217. Kim JH, Kwon SY, Lee SW, Lee CH. Validation of fatty liver index and lipid accumulation product for predicting fatty liver in Korean population. Liver international: official journal of the International Association for the Study of the Liver. 2011;31(10):1600-1.

218. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC gastroenterology. 2010;10(1):98.

219. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137(3):865-72.

220. Ratziu V, Ghabril M, Romero-Gomez M, Svegliati-Baroni GJT. Recommendations for management and treatment of nonalcoholic steatohepatitis. 2019;103(1):28-38.

221. Chaves GV, de Souza DS, Pereira SE, Saboya CJ, Peres WAF. Association between non-alcoholic fatty liver disease and liver function/injury markers with metabolic syndrome components in class III obese individuals. Revista da Associação Médica Brasileira (English Edition). 2012;58(3):288-93.

222. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. Clinical Gastroenterology and Hepatology. 2008;6(12):1396-402.

223. Lee Y, Doumouras AG, Yu J, Brar K, Banfield L, Gmora S, et al. Complete resolution of nonalcoholic fatty liver disease after bariatric surgery: a systematic review and meta-analysis. Clinical Gastroenterology and Hepatology. 2019;17(6):1040-60. e11.

224. Fakhry TK, Mhaskar R, Schwitalla T, Muradova E, Gonzalvo JP, Murr MM. Bariatric surgery improves nonalcoholic fatty liver disease: a contemporary systematic review and meta-analysis. Surgery for Obesity and Related Diseases. 2019;15(3):502-11.

225. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005-23.

226. Watanabe S, Hashimoto E, Ikejima K, Uto H, Ono M, Sumida Y, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Journal of gastroenterology. 2015;50(4):364-77.

227. Sumida Y, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. Journal of gastroenterology. 2018;53(3):362-76.

228. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. New England Journal of Medicine. 2010;362(18):1675-85.

229. Sato K, Gosho M, Yamamoto T, Kobayashi Y, Ishii N, Ohashi T, et al. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Nutrition. 2015;31(7-8):923-30.

230. Miller III ER, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Annals of internal medicine. 2005;142(1):37-46.

231. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Jama. 2009;301(1):39-51.

232. Schürks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. Bmj. 2010;341.

233. Sumida Y, Seko Y, Yoneda M, NAFLD JSGo. Novel antidiabetic medications for non-alcoholic fatty liver disease with type 2 diabetes mellitus. Hepatology research. 2017;47(4):266-80.

234. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65(5):1557-65.

235. Lee C-H, Fu Y, Yang S-J, Chi C-C. Effects of omega-3 polyunsaturated fatty acid supplementation on non-alcoholic fatty liver: A systematic review and meta-analysis. Nutrients. 2020;12(9):2769.

236. Guo X-f, Wang C, Yang T, Li S, Li K-l, Li D. Vitamin D and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. Food & Function. 2020;11(9):7389-99.

237. Xiao M-W, Lin S-X, Shen Z-H, Luo W-W, Wang X-Y. Systematic review with meta-analysis: The effects of probiotics in nonalcoholic fatty liver disease. Gastroenterology research and practice. 2019;2019.

238. Paris T, George ES, Roberts SK, Tierney AC. The effects of diet and lifestyle interventions on insulin resistance in patients with nonalcoholic fatty liver disease: a systematic review. European journal of gastroenterology & hepatology. 2017;29(8):867-78.

239. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. The American journal of clinical nutrition. 2007;86(2):285-300.

240. Kenneally S, Sier JH, Moore JB. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. BMJ open gastroenterology. 2017;4(1).

241. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology. 2015;149(2):367-78. e5.

242. Munteanu MA, Nagy GA, Mircea PA. Current management of NAFLD. Clujul medical. 2016;89(1):19.

243. Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. Liver International. 2017;37(7):936-49.

244. Chait A, den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. Frontiers in cardiovascular medicine. 2020;7.

245. Musso G, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, et al. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. Hepatology. 2003;37(4):909-16.

246. George ES, Tierney AC, Campbell KL, Macdonald GA, Hickman IJ. What is the optimal dietary composition for NAFLD? Current Hepatology Reports. 2017;16(4):346-55.

247. Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. Journal of the American College of Cardiology. 2006;48(4):677-85.

248. Conlon BA, Beasley JM, Aebersold K, Jhangiani SS, Wylie-Rosett J. Nutritional management of insulin resistance in nonalcoholic fatty liver disease (NAFLD). Nutrients. 2013;5(10):4093-114.

249. Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. Diabetes, metabolic syndrome and obesity: targets and therapy. 2019;12:2221.

250. Caporaso N, Morisco F, Camera S, Graziani G, Donnarumma L, Ritieni A. Dietary approach in the prevention and treatment of NAFLD. Front Biosci. 2012;17:2259-68.

251. Finelli C, Tarantino G. Is there any consensus as to what diet or lifestyle approach is the right one for NAFLD patients. J Gastrointestin Liver Dis. 2012;21(3):293-302.

252. Zelber-Sagi S, Godos J, Salomone F. Lifestyle changes for the treatment of nonalcoholic fatty liver disease: a review of observational studies and intervention trials. Therapeutic advances in gastroenterology. 2016;9(3):392-407.

253. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation. 2000;102(18):2284-99.

254. Thomas GN, Ho S-Y, Janus ED, Lam KS, Hedley AJ, Lam TH, et al. The US national cholesterol education programme adult treatment panel III (NCEP ATP III) prevalence of the metabolic syndrome in a Chinese population. Diabetes research and clinical practice. 2005;67(3):251-7.

255. Razavi Zade M, Telkabadi MH, Bahmani F, Salehi B, Farshbaf S, Asemi Z. The effects of DASH diet on weight loss and metabolic status in adults with non-alcoholic fatty liver disease: a randomized clinical trial. Liver International. 2016;36(4):563-71.

256. Reddy AJ, George ES, Roberts SK, Tierney AC. Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with nonalcoholic fatty liver disease: a systematic literature review. Nutrition reviews. 2019;77(11):765-86.

257. Malaguarnera M, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, et al. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. The American journal of gastroenterology. 2010;105(6):1338.

258. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Digestive diseases and sciences. 2012;57(2):545-53.

259. Vollmer WM, Sacks FM, Ard J, Appel LJ, Bray GA, Simons-Morton DG, et al. Effects of diet and sodium intake on blood pressure: subgroup analysis of the DASH-sodium trial. Annals of internal medicine. 2001;135(12):1019-28.

260. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. New England journal of medicine. 2001;344(1):3-10.

261. Azadbakht L, Fard NRP, Karimi M, Baghaei MH, Surkan PJ, Rahimi M, et al. Effects of the Dietary Approaches to Stop Hypertension (DASH) eating plan on cardiovascular risks among type 2 diabetic patients: a randomized crossover clinical trial. Diabetes care. 2011;34(1):55-7.

262. Campbell AP. DASH eating plan: an eating pattern for diabetes management. Diabetes Spectrum. 2017;30(2):76-81.

263. Maskarinec G, Lim U, Jacobs S, Monroe KR, Ernst T, Buchthal SD, et al. Diet quality in midadulthood predicts visceral adiposity and liver fatness in older ages: the Multiethnic Cohort Study. Obesity. 2017;25(8):1442-50.

264. Park S-Y, Noureddin M, Boushey C, Wilkens LR, Setiawan VW. Diet quality association with nonalcoholic fatty liver disease by cirrhosis status: The multiethnic cohort. Current developments in nutrition. 2020;4(3):nzaa024.

265. Watzinger C, Nonnenmacher T, Grafetstätter M, Sowah SA, Ulrich CM, Kauczor H-U, et al. Dietary Factors in Relation to Liver Fat Content: A Cross-sectional Study. Nutrients. 2020;12(3):825.

266. Kwan MW-M, Wong MC-S, Wang HH-X, Liu KQ-L, Lee CL-S, Yan BP-Y, et al. Compliance with the Dietary Approaches to Stop Hypertension (DASH) diet: a systematic review. PLoS One. 2013;8(10):e78412.

267. York LW, Puthalapattu S, Wu GY. Nonalcoholic fatty liver disease and low-carbohydrate diets. Annual Review of Nutrition. 2009;29:365-79.

268. Haghighatdoost F, Salehi-Abargouei A, Surkan PJ, Azadbakht L. The effects of low carbohydrate diets on liver function tests in nonalcoholic fatty liver disease: A systematic review and meta-analysis of clinical trials. Journal of research in medical sciences: The official journal of Isfahan University of Medical Sciences. 2016;21.

269. Huang MA, Greenson JK, Chao C, Anderson L, Peterman D, Jacobson J, et al. One-year intense nutritional counseling results in histological improvement in patients with nonalcoholic steatohepatitis: a pilot study. The American Journal of Gastroenterology. 2005;100(5):1072.

270. Ryan MC, Abbasi F, Lamendola C, Carter S, McLaughlin TL. Serum alanine aminotransferase levels decrease further with carbohydrate than fat restriction in insulin-resistant adults. Diabetes Care. 2007;30(5):1075-80.

271. Golay A, Eigenheer C, Morel Y, Kujawski P, Lehmann T. Weight-loss with low or high carbohydrate diet? International journal of obesity related metabolic disorders: journal of the International Association for the Study of Obesity. 1996;20(12):1067-72.

272. Council NHaMR. Australian Dietary Guidelines. Canberra. 2013.

273. Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, et al. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. Hepatology. 2011;53(5):1504-14.

274. De Luis D, Aller R, Izaola O, Sagrado MG, Conde R. Effect of two different hypocaloric diets in transaminases and insulin resistance in nonalcoholic fatty liver disease and obese patients. Nutricion hospitalaria. 2010;25(5):730-5.

275. Properzi C, O'Sullivan TA, Sherriff JL, Ching HL, Jeffrey GP, Buckley RF, et al. Ad Libitum Mediterranean and Low-Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial. Hepatology. 2018;68(5):1741-54.

276. Abenavoli L, Boccuto L, Federico A, Dallio M, Loguercio C, Di Renzo L, et al. Diet and non-alcoholic fatty liver disease: the Mediterranean way. International journal of environmental research and public health. 2019;16(17):3011.

277. Papamiltiadous ES, Roberts SK, Nicoll AJ, Ryan MC, Itsiopoulos C, Salim A, et al. A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA): study protocol. 2016;16(1):14.

278. Trovato FM, Catalano D, Martines GF, Pace P, Trovato GM. Mediterranean diet and non-alcoholic fatty liver disease: the need of extended and comprehensive interventions. Clinical nutrition. 2015;34(1):86-8.

279. Keys A, Mienotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, et al. The diet and 15-year death rate in the seven countries study. American Journal of Epidemiology. 1986;124(6):903-15.

280. Martínez-González MÁ, Hershey MS, Zazpe I, Trichopoulou A. Transferability of the Mediterranean diet to non-Mediterranean countries. What is and what is not the Mediterranean diet. Nutrients. 2017;9(11):1226.

281. Trichopoulou A, Lagiou P. Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. Nutrition reviews. 1997;55(11):383-9.

282. Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, et al. Mediterranean diet pyramid: a cultural model for healthy eating. The American journal of clinical nutrition. 1995;61(6):1402S-6S.

283. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. Public health nutrition. 2011;14(12A):2274-84.

284. Davis C, Bryan J, Hodgson J, Murphy K. Definition of the Mediterranean diet; a literature review. Nutrients. 2015;7(11):9139-53.

285. Suárez M, Boqué N, del Bas J, Mayneris-Perxachs J, Arola L, Caimari AJN. Mediterranean diet and multi-ingredient-based interventions for the management of non-alcoholic fatty liver disease. 2017;9(10):1052.

286. Vallverdú-Queralt A, de Alvarenga JFR, Estruch R, Lamuela-Raventos RM. Bioactive compounds present in the Mediterranean sofrito. Food chemistry. 2013;141(4):3365-72.

287. Pérez-Guisado J, Muñoz-Serrano A. The effect of the Spanish Ketogenic Mediterranean Diet on nonalcoholic fatty liver disease: a pilot study. Journal of medicinal food. 2011;14(7-8):677-80.

288. Gelli C, Tarocchi M, Abenavoli L, Di Renzo L, Galli A, De Lorenzo A. Effect of a counseling-supported treatment with the Mediterranean diet and physical activity on the severity of the non-alcoholic fatty liver disease. World journal of gastroenterology. 2017;23(17):3150.

289. Kontogianni MD, Tileli N, Margariti A, Georgoulis M, Deutsch M, Tiniakos D, et al. Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease. Clinical Nutrition. 2014;33(4):678-83.

290. Abenavoli L, Greco M, Milic N, Accattato F, Foti D, Gulletta E, et al. Effect of Mediterranean Diet and Antioxidant Formulation in Non-Alcoholic Fatty Liver Disease: A Randomized Study. Nutrients. 2017;9(8):870.

291. Misciagna G, Del Pilar Diaz M, Caramia D, Bonfiglio C, Franco I, Noviello M, et al. Effect of a low glycemic index Mediterranean diet on non-alcoholic fatty liver disease. A randomized controlled clinici trial. The journal of nutrition, health & aging. 2017;21(4):404-12.

292. Katsagoni CN, Papatheodoridis GV, Ioannidou P, Deutsch M, Alexopoulou A, Papadopoulos N, et al. Improvements in clinical characteristics of patients with non-alcoholic fatty liver disease, after an intervention based on the Mediterranean lifestyle: a randomised controlled clinical trial. British Journal of Nutrition. 2018;120(2):164-75.

293. Gepner Y, Shelef I, Komy O, Cohen N, Schwarzfuchs D, Bril N, et al. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content. Journal of hepatology. 2019;71(2):379-88.

294. Gepner Y, Shelef I, Komy O, Cohen N, Schwarzfuchs D, Bril N, et al. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content. Journal of hepatology. 2019.

295. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. Journal of Hepatology. 2013;59(1):138-43.

296. Tarantino G, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. World journal of gastroenterology: WJG. 2010;16(38):4773.

297. Deibert P, Lazaro A, Schaffner D, Berg A, Koenig D, Kreisel W, et al. Comprehensive lifestyle intervention vs soy protein-based meal regimen in non-alcoholic steatohepatitis. World Journal of Gastroenterology. 2019;25(9):1116-31.

298. Rezaei S, Sasani MR, Akhlaghi M, Kohanmoo A. Flaxseed oil in the context of a weight loss programme ameliorates fatty liver grade in patients with non-alcoholic fatty liver disease: a randomised double-blind controlled trial. British Journal of Nutrition. 2020;123(9):994-1002.

299. Behrouz V, Aryaeian N, Zahedi MJ, Jazayeri S. Effects of probiotic and prebiotic supplementation on metabolic parameters, liver aminotransferases, and systemic inflammation in nonalcoholic fatty liver disease: A randomized clinical trial. Journal of Food Science. 2020;85(10):3611-7.

300. Darand M, Darabi Z, Yari Z, Saadati S, Hedayati M, Khoncheh A, et al. Nigella sativa and inflammatory biomarkers in patients with non-alcoholic fatty liver disease: Results from a randomized, double-blind, placebo-controlled, clinical trial. Complementary Therapies in Medicine. 2019;44:204-9.

301. Duseja A, Acharya SK, Mehta M, Chhabra S, Shalimar, Rana S, et al. High potency multistrain probiotic improves liver histology in non-alcoholic fatty liver disease (NAFLD): a randomised, double-blind, proof of concept study. BMJ Open Gastroenterology. 2019;6(1):e000315.

302. Kazemi S, Shidfar F, Ehsani S, Adibi P, Janani L, Eslami O. The effects of sumac (Rhus coriaria L.) powder supplementation in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Complementary Therapies in Clinical Practice. 2020;41:101259.

303. Rafie R, Hosseini SA, Hajiani E, Saki Malehi A, Mard SA. Effect of Ginger Powder Supplementation in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial. Clinical & Experimental Gastroenterology. 2020;13:35-45.

304. Haidari F, Hojhabrimanesh A, Helli B, Seyedian SS, Ahmadi-Angali K. An energy-restricted high-protein diet supplemented with beta-cryptoxanthin alleviated oxidative stress and inflammation in nonalcoholic fatty liver disease: a randomized controlled trial. Nutrition Research. 2020;73:15-26.

305. Yari Z, Cheraghpour M, Alavian SM, Hedayati M, Eini-Zinab H, Hekmatdoost A. The efficacy of flaxseed and hesperidin on non-alcoholic fatty liver disease: an open-labeled randomized controlled trial. European Journal of Clinical Nutrition. 2020;09:09.

306. Abenavoli L, Di Renzo L, Boccuto L, Alwardat N, Gratteri S, De Lorenzo A. Health benefits of Mediterranean diet in nonalcoholic fatty liver disease. Expert Review of Gastroenterology & Hepatology. 2018;12(9):873-81.

307. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World journal of gastroenterology. 2018;24(30):3361.

308. Cerletti C, Colucci M, Storto M, Semeraro F, Ammollo CT, Incampo F, et al. Randomised trial of chronic supplementation with a nutraceutical mixture in subjects with non-alcoholic fatty liver disease. British Journal of Nutrition. 2020;123(2):190-7.

309. Obesity NAAftSo, Heart N, Lung, Institute B, Initiative NOE. The practical guide: identification, evaluation, and treatment of overweight and obesity in adults: National Institutes of Health, National Heart, Lung, and Blood Institute …; 2000.

310. Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutrition reviews. 2013;71(8):511-27.

311. Schwingshackl L, Missbach B, König J, Hoffmann G. Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis. Public health nutrition. 2015;18(7):1292-9.

312. Estruch R. Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study. Proceedings of the Nutrition Society. 2010;69(3):333-40.

313. Kaliora AC, Gioxari A, Kalafati IP, Diolintzi A, Kokkinos A, Dedoussis GV. The Effectiveness of Mediterranean Diet in Nonalcoholic Fatty Liver Disease Clinical Course: An Intervention Study. Journal of medicinal food. 2019;22(7):729-40.

314. Salas-Salvadó J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, et al. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. European journal of clinical nutrition. 2008;62(5):651-9.

315. Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition. 2006;96(S2):S52-S60.

316. Salas-Salvadó J, Bulló M, Pérez-Heras A, Ros E. Dietary fibre, nuts and cardiovascular diseases. British Journal of Nutrition. 2006;96(S2):S45-S51.

317. Jiang R, Jacobs Jr DR, Mayer-Davis E, Szklo M, Herrington D, Jenny NS, et al. Nut and seed consumption and inflammatory markers in the multi-ethnic study of atherosclerosis. American journal of epidemiology. 2006;163(3):222-31.

318. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary α-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. The Journal of nutrition. 2004;134(11):2991-7.

319. Ortega R. Importance of functional foods in the Mediterranean diet. Public health nutrition. 2006;9(8A):1136-40.

320. Bonaccio M, Pounis G, Cerletti C, Donati MB, Iacoviello L, de Gaetano G. Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study. British journal of clinical pharmacology. 2017;83(1):107-13.

321. Watzl B, Kulling SE, Möseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. The American journal of clinical nutrition. 2005;82(5):1052-8.

322. Wannamethee SG, Lowe GD, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. The American journal of clinical nutrition. 2006;83(3):567-74.

323. He K, Liu K, Daviglus ML, Jenny NS, Mayer-Davis E, Jiang R, et al. Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). The American journal of cardiology. 2009;103(9):1238-43.

324. van Bussel BC, Henry RM, Schalkwijk CG, Ferreira I, Feskens EJ, Streppel MT, et al. Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation during a 6-year follow-up. The Journal of nutrition. 2011;141(9):1719-25.

325. Rahmani S, Sadeghi O, Sadeghian M, Sadeghi N, Larijani B, Esmaillzadeh A. The effect of whole-grain intake on biomarkers of subclinical inflammation: a comprehensive meta-analysis of randomized controlled trials. Advances in Nutrition. 2020;11(1):52-65.

326. Mirmiran P, Amirhamidi Z, Ejtahed H-S, Bahadoran Z, Azizi F. Relationship between diet and non-alcoholic fatty liver disease: a review article. Iranian journal of public health. 2017;46(8):1007.

327. Esposito K, Maiorino MI, Bellastella G, Panagiotakos DB, Giugliano D. Mediterranean diet for type 2 diabetes: cardiometabolic benefits. Endocrine. 2017;56(1):27-32.

328. Mutch DM, Wahli W, Williamson G. Nutrigenomics and nutrigenetics: the emerging faces of nutrition. The FASEB journal. 2005;19(12):1602-16.

329. Farhud D, Yeganeh MZ, Yeganeh MZ. Nutrigenomics and nutrigenetics. Iranian journal of public health. 2010;39(4):1.

330. Anstee QM, Day CP. The genetics of NAFLD. Nature reviews Gastroenterology & hepatology. 2013;10(11):645.

331. Ordovas JM, Corella D, Demissie S, Cupples LA, Couture P, Coltell O, et al. Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. Circulation. 2002;106(18):2315-21.

332. Santos JL, Boutin P, Verdich C, Holst C, Larsen LH, Toubro S, et al. Genotype-by-nutrient interactions assessed in European obese women. European journal of nutrition. 2006;45(8):454-62.

333. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. The American journal of clinical nutrition. 2010;91(3):535-46.

334. Eslam M, George J. Genetic and epigenetic mechanisms of NASH. Hepatol Int. 2016;10(3):394-406.

335. Zaragoza-Martí A, Cabañero-Martínez MJ, Hurtado-Sánchez JA, Laguna-Pérez A, Ferrer-Cascales R. Evaluation of Mediterranean diet adherence scores: a systematic review. BMJ open. 2018;8(2):e019033.

336. Bergmans RS, Malecki KM. The association of dietary inflammatory potential with depression and mental well-being among US adults. Preventive medicine. 2017;99:313-9.

337. Cavicchia PP, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A new dietary inflammatory index predicts interval changes in serum high-sensitivity C-reactive protein. The Journal of Nutrition. 2009;139(12):2365-72.

338. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public health nutrition. 2014;17(8):1689-96.

339. Shivappa N, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, et al. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). Public health nutrition. 2014;17(8):1825-33.

340. Tabung FK, Steck SE, Zhang J, Ma Y, Liese AD, Agalliu I, et al. Construct validation of the dietary inflammatory index among postmenopausal women. Annals of epidemiology. 2015;25(6):398-405.

341. Wood LG, Shivappa N, Berthon BS, Gibson PG, Hebert JR. Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. Clinical Experimental Allergy. 2015;45(1):177-83.

342. Julia C, Assmann KE, Shivappa N, Hebert JR, Wirth MD, Hercberg S, et al. Long-term associations between inflammatory dietary scores in relation to long-term C-reactive protein status measured 12 years later: findings from the Supplementation en Vitamines et Mineraux Antioxydants (SU. VI. MAX) cohort. British Journal of Nutrition. 2017;117(2):306-14.

343. Cantero I, Abete I, Babio N, Arós F, Corella D, Estruch R, et al. Dietary Inflammatory Index and liver status in subjects with different adiposity levels within the PREDIMED trial. Clinical nutrition. 2018;37(5):1736-43.

344. Mazidi M, Shivappa N, Wirth MD, Hebert JR, Kengne AP. Diet with greater inflammatory potential is associated with higher prevalence of fatty liver among US adults. European journal of clinical nutrition. 2018:1.

345. Papamiltiadous ES, Roberts SK, Nicoll AJ, Ryan MC, Itsiopoulos C, Salim A, et al. A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA): study protocol. BMC gastroenterology. 2016;16(1):14.

346. Keys A, Menotti A, Aravanis C, Blackburn H, Djordevič BS, Buzina R, et al. The seven countries study: 2,289 deaths in 15 years. Preventive medicine. 1984;13(2):141-54.

347. Health Mo, Welfare SSHCJAoHM. Dietary guidelines for adults in Greece. 1999;16(5):516-24.

348. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. New England Journal of Medicine. 2013;368(14):1279-90.

349. Itsiopoulos C, Brazionis L, Kaimakamis M, Cameron M, Best JD, O'Dea K, et al. Can the Mediterranean diet lower HbA1c in type 2 diabetes? Results from a randomized cross-over study. Nutrition, Metabolism Cardiovascular Diseases. 2011;21(9):740-7.

350. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. Journal of hepatology. 2013;59(1):138-43.

351. Vincent-Baudry S, Defoort C, Gerber M, Bernard M-C, Verger P, Helal O, et al. The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. The American journal of clinical nutrition. 2005;82(5):964-71.

352. Council. NHaMR. Australian guidelines to reduce health risks from drinking alcohol. NHMRC Canberra. 2009.

353. Crichton GE, Howe PR, Buckley JD, Coates AM, Murphy KJ, Bryan J. Long-term dietary intervention trials: critical issues and challenges. Trials. 2012;13(1):111.

354. VanWormer JJ, Boucher JL. Motivational interviewing and diet modification: a review of the evidence. The Diabetes Educator. 2004;30(3):404-19.

355. Opie RS, O'Neil A, Jacka FN, Pizzinga J, Itsiopoulos C. A modified Mediterranean dietary intervention for adults with major depression: Dietary protocol and feasibility data from the SMILES trial. Nutritional Neuroscience. 2018;21(7):487-501.

356. Council NHaMR. *Australian Dietary Guidelines.* Canberra: National Health and Medical Research Council. 2013.

357. Gabel L, Ridgers ND, Della Gatta P, Arundell L, Cerin E, Robinson S, et al. Associations of sedentary time patterns and TV viewing time with inflammatory and endothelial function biomarkers in children. Pediatric obesity. 2016;11(3):194-201.

358. ISAK. International Standards for Anthropometric Assessment Underdale S. International Society for the Advancement of Kinanthropometry. 2001(Available from: http://www.ceap.br/material/MAT17032011184632.pdf.).

359. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. The Journal of nutrition. 2011;141(6):1140-5.

360. Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PloS one. 2012;7(8):e43134.

361. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. American journal of epidemiology. 1985;122(1):51-65.

362. Helmerhorst HHJ, Brage S, Warren J, Besson H, Ekelund U. A systematic review of reliability and objective criterion-related validity of physical activity questionnaires. International Journal of Behavioral Nutrition and Physical Activity. 2012;9(1):103.

363. McHorney CA, Ware Jr JE, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. Medical care. 1993:247-63.

364. Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. 2012;7(8):e43134.

365. Mayr HL, Thomas CJ, Tierney AC, Kucianski T, George ES, Ruiz-Canela M, et al. Randomization to 6-month Mediterranean diet compared with a low-fat diet leads to improvement in Dietary Inflammatory Index scores in patients with coronary heart disease: the AUSMED Heart Trial. Nutrition research. 2018;55:94-107.

366. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. 2002;35(2):367-72.

367. Tolman KG, Dalpiaz ASJT, management cr. Treatment of non-alcoholic fatty liver disease. 2007;3(6):1153.

368. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui CJJoh. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. 2016;65(3):589-600.

369. Lonardo A, Sookoian S, Pirola CJ, Targher GJM. Non-alcoholic fatty liver disease and risk of cardiovascular disease. 2016;65(8):1136-50.

370. Byrne CD, Targher GJJoh. NAFLD: a multisystem disease. 2015;62(1):S47-S64.

371. Rafiq N, Younossi ZM. Nonalcoholic fatty liver disease: a practical approach to evaluation and management. Clinics in liver disease. 2009;13(2):249-66.

372. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. 2002;123(1):134-40.

373. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. 2003;37(4):917-23.

374. Blachier M, Leleu H, Peck-Radosavljevic M, Valla D-C, Roudot-Thoraval FJJoh. The burden of liver disease in Europe: a review of available epidemiological data. 2013;58(3):593-608.

375. Page JAB. Nonalcoholic fatty liver disease: the hepatic metabolic syndrome. Journal of the American Academy of Nurse Practitioners. 2012;24(6):345-51.

376. Santos RD, Valenti L, Romeo S. Does nonalcoholic fatty liver disease cause cardiovascular disease? Current knowledge and gaps. Atherosclerosis. 2019.

377. Tilg H, Moschen ARJH. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. 2010;52(5):1836-46.

378. Kaliora AC, Kokkinos A, Diolintzi A, Stoupaki M, Gioxari A, Kanellos PT, et al. The effect of minimal dietary changes with raisins in NAFLD patients with non-significant fibrosis: a randomized controlled intervention. Food Function. 2016;7(11):4533-44.

379. Markova M, Pivovarova O, Hornemann S, Sucher S, Frahnow T, Wegner K, et al. Isocaloric diets high in animal or plant protein reduce liver fat and inflammation in individuals with type 2 diabetes. Gastroenterology. 2017;152(3):571-85. e8.

380. Sofi F, Giangrandi I, Cesari F, Corsani I, Abbate R, Gensini GF, et al. Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. 2010;61(8):792-802.

381. Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, et al. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. Digestive Liver Disease. 2008;40(3):194-9.

382. Adams L, Angulo P. Recent concepts in non-alcoholic fatty liver disease. Diabetic Medicine. 2005;22(9):1129-33.

383. Eguchi Y, Eguchi T, Mizuta T, Ide Y, Yasutake T, Iwakiri R, et al. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. Journal of Gastroenterology and Hepatology. 2006;41(5):462-9.

384. Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, et al. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. Obesity. 2008;16(6):1394-9.

385. Kolak M, Westerbacka J, Velagapudi VR, Wågsäter D, Yetukuri L, Makkonen J, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. Diabetes. 2007;56(8):1960-8.

386. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. 2004;40(6):1387-95.

387. Pan J-J, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. World journal of hepatology. 2014;6(5):274.

388. Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology. 2003;124(1):71-9.

389. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. The American Journal of Gastroenterology. 2003;98(5):960-7.

390. Schneider AL, Lazo M, Selvin E, Clark JM. Racial differences in nonalcoholic fatty liver disease in the US population. Obesity reviews. 2014;22(1):292-9.

391. Papatheodoridis GV, Goulis J, Christodoulou D, Manolakopoulos S, Raptopoulou M, Andrioti E, et al. High prevalence of elevated liver enzymes in blood donors: associations with male gender and central adiposity. European Journal of Gastroenterology and Hepatology. 2007;19(4):281-7.

392. Adejumo AC, Samuel GO, Adegbala OM, Adejumo KL, Ojelabi O, Akanbi O, et al. Prevalence, trends, outcomes, and disparities in hospitalizations for nonalcoholic fatty liver disease in the United States. Annals of Gastroenterology. 2019;32(5):504.

393. Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. American Journal of Physiology-Endocrinology Metabolism. 2002;283(6):E1135-E43.

394. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, et al. Sex differences in NAFLD: state of the art and identification of research gaps. Hepatology. 2019.

395. George ES, Tierney AC, Campbell KL, Macdonald GA, Hickman IJJCHR. What Is the Optimal Dietary Composition for NAFLD? 2017;16(4):346-55.

396. Abdelmalek MF, Suzuki A, Guy C, Unalp-Arida A, Colvin R, Johnson RJ, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. 2010;51(6):1961-71.

397. Collison KS, Saleh SM, Bakheet RH, Al-Rabiah RK, Inglis AL, Makhoul NJ, et al. Diabetes of the liver: the link between nonalcoholic fatty liver disease and HFCS-55. 2009;17(11):2003-13.

398. Vos MBJCoil. Nutrition, nonalcoholic fatty liver disease and the microbiome: recent progress in the field. 2014;25(1):61.

399. Toshimitsu K, Matsuura B, Ohkubo I, Niiya T, Furukawa S, Hiasa Y, et al. Dietary habits and nutrient intake in non-alcoholic steatohepatitis. Nutrition. 2007;23(1):46-52.

400. Organization WH. Physical status: The use of and interpretation of anthropometry, Report of a WHO Expert Committee. 1995.

401. Chumlea WC, Guo SS, Kuczmarski RJ, Flegal KM, Johnson CL, Heymsfield SB, et al. Body composition estimates from NHANES III bioelectrical impedance data. International journal of obesity. 2002;26(12):1596.

402. Thomas GN, Ho S-Y, Janus ED, Lam KS, Hedley AJ, Lam TH, et al. The US national cholesterol education programme adult treatment panel III (NCEP ATP III) prevalence of the metabolic syndrome in a Chinese population. 2005;67(3):251-7.

403. Kemp W, Roberts S. FibroScan and transient elastography. Australian Family Physician. 2013;42:468-71.

404. NHMRC. Nutrient Reference Values for Australia and New Zealand; Including Recommended Dietary Intakes. Canberra: Commenwealth of Australia. 2006.

405. Howe P, Meyer B, Record S, Baghurst K. Contribution of red meat to very long chain omega-3 fatty acid (VLCOmega3) intake. Asia Pac J Clin Nutr. 2003;12:S27.

406. Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain ω-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition. 2006;22(1):47-53.

407. Sookoian S, Pirola CJJAp, therapeutics. Systematic review with meta-analysis: risk factors for non-alcoholic fatty liver disease suggest a shared altered metabolic and cardiovascular profile between lean and obese patients. 2017;46(2):85-95.

408. Wong VWS, Hui AY, Tsang SWC, Chan JLY, Tse AML, Chan KF, et al. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. 2006;4(9):1154-61.

409. Chen SH, He F, Zhou HL, Wu HR, Xia C, Li YM. Relationship between nonalcoholic fatty liver disease and metabolic syndrome. Journal of Digestive Diseases. 2011;12(2):125-30.

410. Juneja A. Non-alcoholic fatty liver disease (NAFLD)—the hepatic component of metabolic syndrome. JAPI. 2009;57:201.

411. Streba LAM, Carstea D, Mitruţ P, Vere C, Dragomir N, Streba C. Nonalcoholic fatty liver disease and metabolic syndrome: a concise review. 2008;49(1):13-20.

412. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovascular Diabetology. 2018;17(1):122.

413. Tsochatzis EA, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. Mediators of inflammation. 2009;2009.

414. Aller R, de Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, et al. Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. 2008;53(4):1088-92.

415. Bekaert M, Verhelst X, Geerts A, Lapauw B, Calders PJOR. Association of recently described adipokines with liver histology in biopsy-proven non-alcoholic fatty liver disease: a systematic review. 2016;17(1):68-80.

416. Jamali R, Arj A, Razavizade M, Aarabi MHJM. Prediction of nonalcoholic fatty liver disease via a novel panel of serum adipokines. 2016;95(5).

417. Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, et al. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. 2006;91(3):1081-6.

418. Perseghin G, Lattuada G, De Cobelli F, Ntali G, Esposito A, Burska A, et al. Serum resistin and hepatic fat content in nondiabetic individuals. 2006;91(12):5122-5.

419. Cho YK, Lee WY, Oh SY, Park JH, Kim HJ, Park DI, et al. Factors affecting the serum levels of adipokines in Korean male patients with nonalcoholic fatty liver disease. 2007;54(77):1512-6.

420. Abiru S, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, et al. Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. 2006;26(1):39-45.

421. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras JJM. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. 2011;60(3):313-26.

422. Mayr HL, Tierney AC, Thomas CJ, Ruiz-Canela M, Radcliffe J, Itsiopoulos CJNR. Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis. 2018;50:10-24.

423. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJJH. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. 2003;38(2):413-9.

424. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski AJTJoI. Resistin, an adipokine with potent proinflammatory properties. 2005;174(9):5789-95.

425. Chitturi S, Farrell GC, editors. Etiopathogenesis of nonalcoholic steatohepatitis. Seminars in liver disease; 2001: Copyright© 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….

426. Kumar R, Prakash S, Chhabra S, Singla V, Madan K, Gupta SD, et al. Association of pro-inflammatory cytokines, adipokines & oxidative stress with insulin resistance & non-alcoholic fatty liver disease. The Indian journal of medical research. 2012;136(2):229.

427. Inadera H. The usefulness of circulating adipokine levels for the assessment of obesity-related health problems. International journal of medical sciences. 2008;5(5):248.

428. Bugianesi E, Gentilcore E, Vanni E, Rizzetto M, Cassader M, Pasquali R, et al. Plasma Adiponectin in Nonalcoholic Fatty Liver Is Related to Hepatic Insulin Resistance and Hepatic Fat Content, Not to Liver Disease Severity. The Journal of Clinical Endocrinology & Metabolism. 2005;90(6):3498-504.

429. Targher G, Bertolini L, Rodella S, Zoppini G, Scala L, Zenari L, et al. Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. Clinical Endocrinology. 2006;64(6):679-83.

430. Lawlor DA, Davey Smith G, Ebrahim S, Thompson C, Sattar N. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. The Journal of Clinical Endocrinology & Metabolism. 2005;90(10):5677-83.

431. Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, et al. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. European heart journal. 2006;27(19):2300-9.

432. Laughlin GA, Barrett-Connor E, May S, Langenberg C. Association of adiponectin with coronary heart disease and mortality: the Rancho Bernardo study. American journal of epidemiology. 2006;165(2):164-74.

433. Kistorp C, Faber, J., Galatius, S., Gustafsson, F., Frystyk, J., Flyvbjerg, A., & Hildebrandt, P. . Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation. 2005;112(12):1756-62.

434. Pilz S, Mangge H, Wellnitz B, Seelhorst U, Boehm BO, Tiran B, et al. Adiponectin and Mortality in Patients Undergoing Coronary Angiography. The Journal of Clinical Endocrinology & Metabolism. 2006;91(11):4277-86.

435. Argentou M, Tiniakos DG, Karanikolas M, Melachrinou M, Makri MG, Kittas C, et al. Adipokine serum levels are related to liver histology in severely obese patients undergoing bariatric surgery. 2009;19(9):1313-23.

436. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Häkkinen A-M, et al. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia. 2004;47(8):1360-9.

437. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity reviews. 2010;11(1):11-8.

438. Miriam Cnop PH, KM Utzschneider, DB Carr, MK Sinha, EJ Boyko, BM Retzlaff, RH Knopp, JD Brunzell, Steven E Kahn Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(4):459-69.

439. Tsochatzis E, Papatheodoridis GV, Hadziyannis E, Georgiou A, Kafiri G, Tiniakos DG, et al. Serum adipokine levels in chronic liver diseases: association of resistin levels with fibrosis severity. 2008;43(9):1128-36.

440. Ayonrinde OT, Olynyk JK, Beilin LJ, Mori TA, Pennell CE, de Klerk N, et al. Gender-specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. Hepatology. 2011;53(3):800-9.

441. Hashimoto E, Tokushige KJJog. Prevalence, gender, ethnic variations, and prognosis of NASH. 2011;46(1):63-9.

442. Yatsuji S, Hashimoto E, Tobari M, Tokushige K, Shiratori KJHr. Influence of age and gender in Japanese patients with non-alcoholic steatohepatitis. 2007;37(12):1034-43.

443. Gutierrez-Grobe Y, Ponciano-Rodríguez G, Ramos MH, Uribe M, Méndez-Sánchez NJAH. Prevalence of non alcoholic fatty liver disease in premenopausal, posmenopausal and polycystic ovary syndrome women. The role of estrogens. 2010;9(4):402-9.

444. Farrell GC, Wong VW-S, Chitturi S. NAFLD in Asia—as common and important as in the West. Nature reviews Gastroenterology & hepatology. 2013;10(5):307.

445. Weston SR, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. Hepatology. 2005;41(2):372-9.

446. Bambha K, Belt P, Abraham M, Wilson LA, Pabst M, Ferrell L, et al. Ethnicity and nonalcoholic fatty liver disease. Hepatology. 2012;55(3):769-80.

447. Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH, et al. Prevalence and etiology of elevated serum alanine aminotransferase level in an adult population in Taiwan. Journal of gastroenterology and hepatology. 2007;22(9):1482-9.

448. Fan J-G, Zhu J, Li X-J, Chen L, Lu Y-S, Li L, et al. Fatty liver and the metabolic syndrome among Shanghai adults. Journal of Gastroenterology and Hepatology. 2005;20(12):1825-32.

449. Fan J-G, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. Journal of hepatology. 2009;50(1):204-10.

450. PARK SH, JEON WK, KIM SH, KIM HJ, PARK DI, CHO YK, et al. Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. Journal of gastroenterology and hepatology. 2006;21(1):138-43.

451. Chung GE, Youn J, Kim YS, Lee JE, Yang SY, Lim JH, et al. Dietary patterns are associated with the prevalence of nonalcoholic fatty liver disease in Korean adults. Nutrition. 2019;62:32-8.

452. Panagiotakos DB, Pitsavos C, Yannakoulia M, Chrysohoou C, Stefanadis C. The implication of obesity and central fat on markers of chronic inflammation: The ATTICA study. Atherosclerosis. 2005;183(2):308-15.

453. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. International Journal of Obesity. 2001;25(10):1416.

454. Rexrode KM, Pradhan A, Manson JE, Buring JE, Ridker PM. Relationship of total and abdominal adiposity with CRP and IL-6 in women. Annals of epidemiology. 2003;13(10):674-82.

455. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. Diabetes research clinical practice. 2005;69(1):29-35.

456. Stojsavljević S, Palčić MG, Jukić LV, Duvnjak LS, Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in the pathogenesis of nonalcoholic fatty liver disease. World journal of gastroenterology. 2014;20(48):18070.

457. Maury E, Brichard S. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Molecular Cellular Endocrinology. 2010;314(1):1-16.

458. Paz-Filho G, Mastronardi C, Franco CB, Wang KB, Wong M-L, Licinio J. Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. Arquivos Brasileiros de Endocrinologia Metabologia. 2012;56(9):597-607.

459. Rezaei S, Akhlaghi M, Sasani MR, Boldaji RB. Olive oil lessened fatty liver severity independent of cardiometabolic correction in patients with non-alcoholic fatty liver disease: A randomized clinical trial. Nutrition. 2019;57:154-61.

460. Fitzpatrick E, Dhawan AJWjogW. Noninvasive biomarkers in non-alcoholic fatty liver disease: current status and a glimpse of the future. 2014;20(31):10851.

461. Cortez-Pinto H, Jesus L, Barros H, Lopes C, Moura M, Camilo MJCN. How different is the dietary pattern in non-alcoholic steatohepatitis patients? 2006;25(5):816-23.

462. McCarthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic fatty liver disease. 2012;112(3):401-9.

463. Volynets V, Küper MA, Strahl S, Maier IB, Spruss A, Wagnerberger S, et al. Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). 2012;57(7):1932-41.

464. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, et al. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. 2007;47(5):711-7.

465. Toshimitsu K, Matsuura B, Ohkubo I, Niiya T, Furukawa S, Hiasa Y, et al. Dietary habits and nutrient intake in non-alcoholic steatohepatitis. 2007;23(1):46-52.

466. Slavin JL, Martini MC, Jacobs Jr DR, Marquart LJTAjocn. Plausible mechanisms for the protectiveness of whole grains. 1999;70(3):459s-63s.

467. Della Pepa G, Vetrani C, Lombardi G, Bozzetto L, Annuzzi G, Rivellese AJN. Isocaloric dietary changes and non-alcoholic fatty liver disease in high cardiometabolic risk individuals. 2017;9(10):1065.

468. Alavian SM, Esmaillzadeh A, Adibi P, Azadbakht LJHm. Dietary quality indices and biochemical parameters among patients with non alcoholic fatty liver disease (NAFLD). 2013;13(7).

469. Kobayashi Y, Tatsumi H, Hattori M, Sugiyama H, Wada S, Kuwahata M, et al. Comparisons of dietary intake in Japanese with non-alcoholic fatty liver disease and type 2 diabetes mellitus. 2016:16-7.

470. Abenavoli L, Greco M, Milic N, Accattato F, Foti D, Gulletta E, et al. Effect of Mediterranean diet and antioxidant formulation in non-alcoholic fatty liver disease: A randomized study. 2017;9(8):870.

471. Garcia M, Bihuniak J, Shook J, Kenny A, Kerstetter J, Huedo-Medina TJN. The effect of the traditional Mediterranean-style diet on metabolic risk factors: A meta-analysis. 2016;8(3):168.

472. Neuschwander-Tetri BA, Caldwell SHJH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. 2003;37(5):1202-19.

473. Angulo P. Nonalcoholic fatty liver disease. New England Journal of Medicine 2002;346(16):1221-31.

474. Eslamparast T, Tandon P, Raman MJN. Dietary composition independent of weight loss in the management of non-alcoholic fatty liver disease. 2017;9(8):800.

475. Loomba R, Sanyal AJJNrG, hepatology. The global NAFLD epidemic. 2013;10(11):686.

476. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. 2015;100(6):2231-8.

477. Yamazaki H, Tsuboya T, Tsuji K, Dohke M, Maguchi HJDC. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. 2015;38(9):1673-9.

478. Vernon G, Baranova A, Younossi ZJAp, therapeutics. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. 2011;34(3):274-85.

479. Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. 2007;30(8):2119-21.

480. Lonardo A, Nascimbeni F, Maurantonio M, Marrazzo A, Rinaldi L, Adinolfi LEJWjog. Nonalcoholic fatty liver disease: Evolving paradigms. 2017;23(36):6571.

481. Buzzetti E, Pinzani M, Tsochatzis EAJM. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). 2016;65(8):1038-48.

482. Tilg H, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends in Endocrinology Metabolism. 2008;19(10):371-9.

483. Ajmal MR, Yaccha M, Malik MA, Rabbani M, Ahmad I, Isalm N, et al. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF-α. Indian heart journal. 2014;66(6):574-9.

484. Richard C, Couture P, Desroches S, Lamarche B. Effect of the Mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity. 2013;21(1):51-7.

485. Milić S, Lulić D, Štimac D. Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations. World journal of Gastroenterology. 2014;20(28):9330.

486. Machado MV, Coutinho J, Carepa F, Costa A, Proença H, Cortez-Pinto H. How adiponectin, leptin, and ghrelin orchestrate together and correlate with the severity of nonalcoholic fatty liver disease. European Journal of Gastroenterology Hepatology. 2012;24(10):1166-72.

487. Buechler C, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. World Journal of Gastroenterology. 2011;17(23):2801.

488. Loffreda S, Yang S, Lin H, Karp C, Brengman ML, Wang D, et al. Leptin regulates proinflammatory immune responses. The FASEB journal. 1998;12(1):57-65.

489. Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, et al. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. The journal of clinical endocrinology metabolism. 2006;91(3):1081-6.

490. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science. 1996;274(5290):1185-8.

491. Cohen B, Novick D, Rubinstein MJS. Modulation of insulin activities by leptin. 1996;274(5290):1185-8.

492. de Oliveira R, Moraes TI, Cerda A, Hirata MH, Fajardo CM, Sousa MC, et al. ADIPOQ and IL6 variants are associated with a pro-inflammatory status in obeses with cardiometabolic dysfunction. Diabetology & Metabolic Syndrome. 2015;7(1):34.

493. Ahluwalia N, Andreeva V, Kesse-Guyot E, Hercberg SJD, metabolism. Dietary patterns, inflammation and the metabolic syndrome. 2013;39(2):99-110.

494. Liver EAftSot, Diabetes EAftSo. EASL-EASD-EASO Clinical Practice Guidelines for t he management of non-alcoholic fatty liver disease. 2016;9(2):65-90.

495. Paris T, George ES, Roberts SK, Tierney AC, hepatology. The effects of diet and lifestyle interventions on insulin resistance in patients with nonalcoholic fatty liver disease: a systematic review. European Journal of Gastroenterology Hepatology. 2017;29(8):867-78.

496. Andersen T, Gluud C, Franzmann M-B, Christoffersen PJJoh. Hepatic effects of dietary weight loss in morbidly obese subjects. 1991;12(2):224-9.

497. McCarthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic fatty liver disease. Journal of the Academy of Nutrition Dietetics. 2012;112(3):401-9.

498. Abenavoli L, Milic N, Peta V, Alfieri F, De Lorenzo A, Bellentani S. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. World Journal of Gastroenterology: WJG. 2014;20(45):16831.

499. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. 2013;59(1):138-43.

500. Kontogianni MD, Tileli N, Margariti A, Georgoulis M, Deutsch M, Tiniakos D, et al. Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease. 2014;33(4):678-83.

501. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292(12):1440-6.

502. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA. 2003;289(14):1799-804.

503. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Annals of Internal Medicine. 2006;145(1):1-11.

504. Mena M-P, Sacanella E, Vazquez-Agell M, Morales M, Fito M, Escoda R, et al. Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. The American journal of clinical nutrition. 2008;89(1):248-56.

505. Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FBJTAjocn. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women–. 2006;84(2):328-35.

506. Koloverou E, Panagiotakos D, Pitsavos C, Chrysohoou C, Georgousopoulou E, Grekas A, et al. Adherence to Mediterranean diet and 10-year incidence (2002–2012) of diabetes:

correlations with inflammatory and oxidative stress biomarkers in the ATTICA cohort study. Diabetes & metabolism research reviews. 2016;32(1):73-81.

507. Mayr HL, Tierney AC, Thomas CJ, Ruiz-Canela M, Radcliffe J, Itsiopoulos C. Mediterranean-type diets and inflammatory markers in patients with coronary heart disease: a systematic review and meta-analysis. Nutrition Research. 2018;50:10-24.

508. Steckhan N, Hohmann C-D, Kessler C, Dobos G, Michalsen A, Cramer H. Effects of different dietary approaches on inflammatory markers in patients with metabolic syndrome: a systematic review and meta-analysis. Nutrition Research. 2016;32(3):338-48.

509. Assy N, Nassar F, Nasser G, Grosovski MJWjogW. Olive oil consumption and non-alcoholic fatty liver disease. 2009;15(15):1809.

510. Fitó M, Cladellas M, De la Torre R, Marti J, Munoz D, Schröder H, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. European journal of clinical nutrition. 2008;62(4):570.

511. Estruch R. Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study. J Proceedings of the Nutrition Society. 2010;69(3):333-40.

512. Yan J-H, Guan B-J, Gao H-Y, Peng X-EJM. Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. 2018;97(37).

513. O'Keefe JH, Gheewala NM, O'Keefe JO. Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. Journal of the American College of Cardiology. 2008;51(3):249-55.

514. Radulian G, Rusu E, Dragomir A, Posea M. Metabolic effects of low glycaemic index diets. Nutrition Journal. 2009;8(1):5.

515. Jenkins D, Wolever T, Kalmusky J, Guidici S, Giordano C, Patten R, et al. Low-glycemic index diet in hyperlipidemia: use of traditional starchy foods. The American journal of clinical nutrition. 1987;46(1):66-71.

516. Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. The American Journal of Clinical Nutrition. 2000;71(6):1455-61.

517. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. New England Journal of Medicine. 2003;348(26):2599-608.

518. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study. Journal of the American College of Cardiology. 2004;44(1):152-8.

519. Dai J, Miller AH, Bremner JD, Goldberg J, Jones L, Shallenberger L, et al. Adherence to the Mediterranean diet is inversely associated with circulating interleukin-6 among middle-aged men: a twin study. Circulation. 2008;117(2):169.

520. Fragopoulou E, Panagiotakos DB, Pitsavos C, Tampourlou M, Chrysohoou C, Nomikos T, et al. The association between adherence to the Mediterranean diet and adiponectin levels among healthy adults: the ATTICA study. The Journal of Nutritional Biochemistry. 2010;21(4):285-9.

521. Fung TT, McCullough ML, Newby P, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction–. The American Journal of Clinical Nutrition. 2005;82(1):163-73.

522. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. Annals of internal medicine. 2010;152(11):726-32.

523. Keys A, Mienotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, et al. The diet and 15-year death rate in the seven countries study. 1986;124(6):903-15.

524. Guebre-Egziabher F, Rabasa-Lhoret R, Bonnet F, Bastard J, Desage M, Skilton M, et al. Nutritional intervention to reduce the n− 6/n− 3 fatty acid ratio increases adiponectin concentration and fatty acid oxidation in healthy subjects. 2008;62(11):1287.

525. Piers L, Walker K, Stoney R, Soares M, O'dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). International journal of obesity. 2002;26(6):814.

526. Gabel L, Ridgers ND, Della Gatta P, Arundell L, Cerin E, Robinson S, et al. Associations of sedentary time patterns and TV viewing time with inflammatory and endothelial function biomarkers in children. 2016;11(3):194-201.

527. Aguinis H, Gottfredson RK, Joo H. Best-practice recommendations for defining, identifying, and handling outliers. Organizational Research Methods. 2013;16(2):270-301.

528. Cohen J. Statistical power analysis for the behavioral sciences, Lawrence Earlbaum Associates Publisher. Hillsdale, NJ. 1988:8-16.

529. George ES, Kucianski T, Mayr HL, Moschonis G, Tierney AC, Itsiopoulos C. A Mediterranean diet model in Australia: strategies for translating the traditional Mediterranean diet into a multicultural setting. Nutrients. 2018;10(4):465.

530. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot NJDr, practice c. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. 2014;105(2):141-50.

531. Tilg H, Hotamisligil GSJG. Nonalcoholic fatty liver disease: cytokine-adipokine interplay and regulation of insulin resistance. 2006;131(3):934-45.

532. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George JJH. Beyond insulin resistance in NASH: TNF-α or adiponectin? 2004;40(1):46-54.

533. Markova M, Pivovarova O, Hornemann S, Sucher S, Frahnow T, Wegner K, et al. Isocaloric diets high in animal or plant protein reduce liver fat and inflammation in individuals with type 2 diabetes. 2017;152(3):571-85. e8.

534. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. 2003;289(14):1799-804.

535. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obesity Research. 2003;11(3):368-76.

536. Siriwardhana N, Kalupahana NS, Cekanova M, LeMieux M, Greer B, Moustaid-Moussa N. Modulation of adipose tissue inflammation by bioactive food compounds. The Journal of Nutritional Biochemistry. 2013;24(4):613-23.

537. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS medicine. 2010;7(3):e1000252.

538. Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, Alagona C, et al. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. Digestive Liver Disease. 2008;40(3):194-9.

539. George ES, Marshall S, Mayr HL, Trakman GL, Tatucu-Babet OA, Lassemillante A-CM, et al. The effect of high-polyphenol extra virgin olive oil on cardiovascular risk factors: A systematic review and meta-analysis. Critical Reviews in Food Science Nutrition Research. 2018:1-24.

540. Perdomo CM, Frühbeck G, Escalada J. Impact of nutritional changes on nonalcoholic fatty liver disease. Nutrients. 2019;11(3):677.

541. de Wit NJ, Afman LA, Mensink M, Müller M. Phenotyping the effect of diet on non-alcoholic fatty liver disease. Journal of Hepatology. 2012;57(6):1370-3.

542. Papageorgiou N, Tousoulis D, Psaltopoulou T, Giolis A, Antoniades C, Tsiamis E, et al. Divergent anti-inflammatory effects of different oil acute consumption on healthy individuals. European journal of clinical nutrition. 2011;65(4):514.

543. Nigam P, Bhatt S, Misra A, Chadha DS, Vaidya M, Dasgupta J, et al. Effect of a 6-month intervention with cooking oils containing a high concentration of monounsaturated fatty acids (olive and canola oils) compared with control oil in male Asian Indians with nonalcoholic fatty liver disease. 2014;16(4):255-61.

544. Abid A, Taha O, Nseir W, Farah R, Grosovski M, Assy N. Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome. Journal of Hepatology. 2009;51(5):918-24.

545. Campos V, Despland C, Brandejsky V, Kreis R, Schneiter P, Chiolero A, et al. Sugar-and artificially sweetened beverages and intrahepatic fat: A randomized controlled trial. 2015;23(12):2335-9.

546. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, et al. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. Journal of Hepatology. 2007;47(5):711-7.

547. Jegatheesan P, De Bandt JP. Fructose and NAFLD: The multifaceted aspects of fructose metabolism. Nutrients. 2017;9(3):230.

548. Mager DR, Iñiguez IR, Gilmour S, Yap JJJoP, Nutrition E. The effect of a low fructose and low glycemic index/load (FRAGILE) dietary intervention on indices of liver function, cardiometabolic risk factors, and body composition in children and adolescents with nonalcoholic fatty liver disease (NAFLD). 2015;39(1):73-84.

549. Jin R, Welsh J, Le N-A, Holzberg J, Sharma P, Martin D, et al. Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. Nutrients. 2014;6(8):3187-201.

550. Abdelmalek MF, Suzuki A, Guy C, Unalp-Arida A, Colvin R, Johnson RJ, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. Hepatology. 2010;51(6):1961-71.

551. Ross AB, Godin J-P, Minehira K, Kirwan JP. Increasing whole grain intake as part of prevention and treatment of nonalcoholic Fatty liver disease. International Journal of Endocrinology. 2013;2013.

552. Bartłomiej S, Justyna R-K, Ewa N. Bioactive compounds in cereal grains–occurrence, structure, technological significance and nutritional benefits–a review. Food Science Technology International. 2012;18(6):559-68.

553. Rahmani S, Sadeghi O, Sadeghian M, Sadeghi N, Larijani B, Esmaillzadeh A. The Effect of Whole-Grain Intake on Biomarkers of Subclinical Inflammation: A Comprehensive Meta-analysis of Randomized Controlled Trials. Advances in Nutrition. 2019.

554. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. The American Journal of Clinical Nutrition. 2004;80(4):1029-35.

555. Sureda A, Bibiloni M, Julibert A, Bouzas C, Argelich E, Llompart I, et al. Adherence to the Mediterranean diet and inflammatory markers. Nutrients. 2018;10(1):62.

556. Hermsdorff HHM, Zulet MA, Abete I, Martínez JA. Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. Endocrine. 2009;36(3):445-51.

557. Richard C, Royer M-M, Couture P, Cianflone K, Rezvani R, Desroches S, et al. Effect of the Mediterranean diet on plasma adipokine concentrations in men with metabolic syndrome. Metabolism. 2013;62(12):1803-10.

558. Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. 2010;51(1):121-9.

559. Thoma C, Day CP, Trenell MIJJoh. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. 2012;56(1):255-66.

560. De Luis D, Aller R, Izaola O, Gonzalez Sagrado M, Conde RJNh. Effect of two different hypocaloric diets in transaminases and insulin resistance in nonalcoholic fatty liver disease and obese patients. 2010;25(5).

561. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. The American Journal of Clinical Nutrition. 2000;72(4):905-11.

562. Parry SA, Hodson L. Influence of dietary macronutrients on liver fat accumulation and metabolism. Journal of Investigative Medicine. 2017;65(8):1102-15.

563. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943-51.

564. Twigg SM, Kamp MC, Davis TM, Neylon EK, Flack JR. Prediabetes: a position statement from the australian diabetes society and australian diabetes educators association. Medical journal of Australia. 2007;186(9):461-5.

565. Kenneally S, Sier JH, Moore JBJBog. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. 2017;4(1):e000139.

566. Chan DC, Watts GF, Gan SK, Ooi EM, Barrett PHR. Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. Diabetes Care. 2010;33(5):1134-9.

567. Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. The American journal of clinical nutrition. 2014;99(3):535-42.

568. Malaguarnera M, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, et al. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. American Journal of Gastroenterology. 2010;105(6):1338-45.

569. Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. British Journal of Nutrition. 2017;117(5):662-8.

570. Kemp W, Roberts SJAfp. FibroScan and transient elastography. 2013;42(7):468.

571. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology. 2019;69(6):2672-82.

572. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology. 2003;37(5):1202-19.

573. Dibba P, Li AA, Perumpail BJ, John N, Sallam S, Shah ND, et al. Emerging therapeutic targets and experimental drugs for the treatment of NAFLD. Diseases. 2018;6(3):83.

574. Solga S, Alkhuraishe AR, Clark JM, Torbenson M, Greenwald A, Diehl AM, et al. Dietary composition and nonalcoholic fatty liver disease. Digestive diseases. 2004;49(10):1578-83.

575. Bian H, Hakkarainen A, Lundbom N, Yki-Järvinen H. Effects of dietary interventions on liver volume in humans. Obesity. 2014;22(4):989-95.

576. Tamura S, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. The Journal of clinical investigation. 2005;115(5):1139-42.

577. Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, et al. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes care. 2011;34(1):14-9.

578. Itsiopoulos C, Brazionis L, Kaimakamis M, Cameron M, Best JD, O'Dea K, et al. Can the Mediterranean diet lower HbA1c in type 2 diabetes? Results from a randomized cross-over study. Nutrition, Metabolism and Cardiovascular Diseases. 2011;21(9):740-7.

579. Ahluwalia N, Andreeva VA, Kesse-Guyot E, Hercberg S. Dietary patterns, inflammation and the metabolic syndrome. Diabetes & metabolism. 2013;39(2):99-110.

580. Galland L. Diet and inflammation. Nutrition in Clinical Practice. 2010;25(6):634-40.

581. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. BMJ. 2008;337:a1344.

582. Segal L, Opie RS. A nutrition strategy to reduce the burden of diet related disease: access to dietician services must complement population health approaches. Frontiers in pharmacology. 2015;6:160.

583. Hodge A, Bassett J, Shivappa N, Hébert J, English D, Giles G, et al. Dietary inflammatory index, Mediterranean diet score, and lung cancer: a prospective study. Cancer Causes Control. 2016;27(7):907-17.

584. Ramallal R, Toledo E, Martínez JA, Shivappa N, Hébert JR, Martínez-González MA, et al. Inflammatory potential of diet, weight gain, and incidence of overweight/obesity: The SUN cohort. Obesity. 2017;25(6):997-1005.

585. Turner-McGrievy GM, Wirth MD, Shivappa N, Wingard EE, Fayad R, Wilcox S, et al. Randomization to plant-based dietary approaches leads to larger short-term improvements in Dietary Inflammatory Index scores and macronutrient intake compared with diets that contain meat. Nutrition research. 2015;35(2):97-106.

586. Tabung FK, Steck SE, Zhang J, Ma Y, Liese AD, Tylavsky FA, et al. Longitudinal changes in the dietary inflammatory index: an assessment of the inflammatory potential of diet over time in postmenopausal women. European journal of clinical nutrition. 2016;70(12):1374.

587. Rubió L, Motilva M-J, Romero M-P. Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. Critical reviews in food science and nutrition. 2013;53(9):943-53.

588. Steck S, Shivappa N, Tabung F, Harmon B, Wirth M, Hurley T, et al. The dietary inflammatory index: a new tool for assessing diet quality based on inflammatory potential. Digest. 2014;49(3):1-10.

589. Neufcourt L, Assmann K, Fezeu L, Touvier M, Graffouillère L, Shivappa N, et al. Prospective association between the dietary inflammatory index and metabolic syndrome: Findings from the SU. VI. MAX study. Nutrition, Metabolism Cardiovascular Diseases. 2015;25(11):988-96.

590. Alkerwi Aa, Shivappa N, Crichton G, Hébert JR. No significant independent relationships with cardiometabolic biomarkers were detected in the Observation of Cardiovascular Risk Factors in Luxembourg study population. Nutrition research. 2014;34(12):1058-65.

591. Carvalho CA, Silva AAM, Assunção MCF, Fonseca PCA, Barbieri MA, Bettiol H, et al. The dietary inflammatory index and insulin resistance or metabolic syndrome in young adults. Nutrition. 2019;58:187-93.

592. Sokol A, Wirth MD, Manczuk M, Shivappa N, Zatonska K, Hurley TG, et al. Association between the dietary inflammatory index, waist-to-hip ratio and metabolic syndrome. Nutrition research. 2016;36(11):1298-303.

593. Wirth MD, Hébert JR, Shivappa N, Hand GA, Hurley TG, Drenowatz C, et al. Anti-inflammatory Dietary Inflammatory Index scores are associated with healthier scores on other dietary indices. Nutrition research. 2016;36(3):214-9.

594. Pimenta AM, Toledo E, Rodriguez-Diez MC, Gea A, Lopez-Iracheta R, Shivappa N, et al. Dietary indexes, food patterns and incidence of metabolic syndrome in a Mediterranean cohort: The SUN project. Clinical nutrition. 2015;34(3):508-14.

595. Sen S, Rifas-Shiman SL, Shivappa N, Wirth MD, Hebert JR, Gold DR, et al. Associations of prenatal and early life dietary inflammatory potential with childhood adiposity and cardiometabolic risk in Project Viva. Pediatric obesity. 2018;13(5):292-300.

596. Colegrave N, Ruxton GD. Statistical model specification and power: recommendations on the use of test-qualified pooling in analysis of experimental data. Proceedings of the Royal Society B: Biological Sciences. 2017;284(1851):20161850.

597. Tobias Ferrer J, Santasusana Riera I, Cuadrench Solórzano M, Gonzalez Cabré M, Girbau Tapias M, Sant Masoliver C. Adherence to the Mediterranean Diet in patients with coronary artery disease. Revista Española de Cardiología (English Edition). 2015;68(1):73-5.

598. Cohen J. Statistical power analysis for the behavioral sciences. Abingdon. United Kingdom: Routledge; 1988.

599. Mayr HL, Itsiopoulos C, Tierney AC, Ruiz-Canela M, Hebert JR, Shivappa N, et al. Improvement in dietary inflammatory index score after 6-month dietary intervention is associated with reduction in interleukin-6 in patients with coronary heart disease: The AUSMED heart trial. Nutrition research. 2018;55:108-21.

600. Ruiz-Canela M, Zazpe I, Shivappa N, Hébert JR, Sánchez-Tainta A, Corella D, et al. Dietary inflammatory index and anthropometric measures of obesity in a population sample at high cardiovascular risk from the PREDIMED (PREvencion con DIeta MEDiterranea) trial. British journal of nutrition. 2015;113(6):984-95.

601. Shivappa N, Wirth MD, Hurley TG, Hébert JR. Association between the dietary inflammatory index (DII) and telomere length and C-reactive protein from the National Health and Nutrition Examination Survey-1999–2002. Molecular nutrition & food research. 2017;61(4):1600630.

602. Wirth M, Burch J, Shivappa N, Violanti JM, Burchfiel CM, Fekedulegn D, et al. Association of a dietary inflammatory index with inflammatory indices and the metabolic

syndrome among police officers. Journal of occupational and environmental medicine/American College of Occupational and Environmental Medicine. 2014;56(9):986.

603. Shivappa N, Hébert JR, Rietzschel ER, De Buyzere ML, Langlois M, Debruyne E, et al. Associations between dietary inflammatory index and inflammatory markers in the Asklepios Study. British Journal of Nutrition. 2015;113(4):665-71.

604. Bodén S, Wennberg M, Van Guelpen B, Johansson I, Lindahl B, Andersson J, et al. Dietary inflammatory index and risk of first myocardial infarction; a prospective population-based study. Nutrition journal. 2017;16(1):21.

605. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine. 1996;334(5):292-5.

606. Iikuni N, Lam K, Queenie L, Lu L, Matarese G, Cava AL. Leptin and inflammation. Current immunology reviews. 2008;4(2):70-9.

607. Muhammad HFL, van Baak MA, Mariman EC, Sulistyoningrum DC, Huriyati E, Lee YY, et al. Dietary inflammatory index score and its association with body weight, blood pressure, lipid profile, and leptin in Indonesian adults. Nutrients. 2019;11(1):148.

608. Camargo-Ramos CM, Correa-Bautista JE, Correa-Rodríguez M, Ramírez-Vélez R. Dietary inflammatory index and cardiometabolic risk parameters in overweight and sedentary subjects. International journal of environmental research and public health. 2017;14(10):1104.

609. Huang X-D, Fan Y, Zhang H, Wang P, Yuan JP, Li M-J, et al. Serum leptin and soluble leptin receptor in non-alcoholic fatty liver disease. World journal of gastroenterology: WJG. 2008;14(18):2888.

610. Kim J-Y, Van De Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. The Journal of clinical investigation. 2007;117(9):2621-37.

611. Bendall C, Mayr H, Opie R, Bes-Rastrollo M, Itsiopoulos C, Thomas C. Central obesity and the Mediterranean diet: A systematic review of intervention trials. Critical reviews in food science and nutrition. 2018;58(18):3070-84.

612. Piers L, Walker K, Stoney R, Soares M, O'dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). International journal of obesity. 2002;26(6):814-21.

613. Mayr HL, Itsiopoulos C, Tierney AC, Kucianski T, Radcliffe J, Garg M, et al. Ad libitum Mediterranean diet reduces subcutaneous but not visceral fat in patients with coronary heart disease: A randomised controlled pilot study. Clinical nutrition ESPEN. 2019;32:61-9.

614. Blüher M, Rudich A, Klöting N, Golan R, Henkin Y, Rubin E, et al. Two patterns of adipokine and other biomarker dynamics in a long-term weight loss intervention. Diabetes care. 2012;35(2):342-9.

615. Vilar-Gomez E, Calzadilla-Bertot L, Wong VW-S, Castellanos M, Aller-de la Fuente R, Metwally M, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. Gastroenterology. 2018;155(2):443-57. e17.

616. Liu J, Xing J, Wang B, Wei C, Yang R, Zhu Y, et al. Correlation Between Adiponectin Gene rs1501299 Polymorphism and Nonalcoholic Fatty Liver Disease Susceptibility: A Systematic Review and Meta-Analysis. Med Sci Monit. 2019;25:1078-86.

617. Struben VMD, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. The American journal of medicine. 2000;108(1):9-13.

618. Dongiovanni P, Valenti L, Rametta R, Daly A, Nobili V, Mozzi E, et al. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. Gut. 2010;59(2):267-73.

619. Valenti L, Canavesi E, Galmozzi E, Dongiovanni P, Rametta R, Maggioni P, et al. Beta-globin mutations are associated with parenchymal siderosis and fibrosis in patients with non-alcoholic fatty liver disease. Journal of hepatology. 2010;53(5):927-33.

620. Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, et al. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. Journal of hepatology. 2012;56(2):448-54.

621. Miele L, Beale G, Patman G, Nobili V, Leathart J, Grieco A, et al. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. Gastroenterology. 2008;135(1):282-91. e1.

622. Day CP. Genetic studies to identify hepatic fibrosis genes and SNPs in human populations. Methods Mol Med. 2005;117:315-31.

623. Yang T-L, Hao R-H, Guo Y, Papasian CJ, Hong-Wen Deng. Genetics of Bone Biology and Skeletal Disease Second Edition ed: Academic Press; 2018.

624. Consortium IH. A haplotype map of the human genome. Nature. 2005;437(7063):1299.

625. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure. 1999;7(2):169-77.

626. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. American Journal of Physiology-Heart and Circulatory Physiology. 2005;288(5):H2031-H41.

627. MacGregor AJ, Gallimore JR, Spector TD, Pepys MB. Genetic effects on baseline values of C-reactive protein and serum amyloid a protein: a comparison of monozygotic and dizygotic twins. Clinical chemistry. 2004;50(1):130-4.

628. Retterstol L, Eikvar L, Berg K. A twin study of C-reactive protein compared to other risk factors for coronary heart disease. Atherosclerosis. 2003;169(2):279-82.

629. Arouca AB, Meirhaeghe A, Dallongeville J, Moreno LA, Lourenço GJ, Marcos A, et al. Interplay between the Mediterranean diet and C-reactive protein genetic polymorphisms towards inflammation in adolescents. Clinical Nutrition. 2019.

630. Nienaber-Rousseau C, Swanepoel B, Dolman RC, Pieters M, Conradie KR, Towers GW. Interactions between C-reactive protein genotypes with markers of nutritional status in relation to inflammation. Nutrients. 2014;6(11):5034-50.

631. Teng M-S, Hsu L-A, Wu S, Chang H-H, Chou H-H, Ko Y-L. Association between C-reactive protein gene haplotypes and C-reactive protein levels in Taiwanese: interaction with obesity. Atherosclerosis. 2009;204(2):e64-e9.

632. Miller D, Zee R, Suk Danik J, Kozlowski P, Chasman D, Lazarus R, et al. Association of common CRP gene variants with CRP levels and cardiovascular events. Annals of human genetics. 2005;69(6):623-38.

633. Berger P, McConnell JP, Nunn M, Kornman KS, Sorrell J, Stephenson K, et al. C-reactive protein levels are influenced by common IL-1 gene variations. Cytokine. 2002;17(4):171-4.

634. Latkovskis G, Licis N, Kalnins U. C-reactive protein levels and common polymorphisms of the interleukin-1 gene cluster and interleukin-6 gene in patients with coronary heart disease. European journal of immunogenetics. 2004;31(5):207-13.

635. Vickers MA, Green FR, Terry C, Mayosi BM, Julier C, Lathrop M, et al. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovascular research. 2002;53(4):1029-34.

636. Goyenechea E, Parra D, Martínez JA. Impact of interleukin 6− 174G> C polymorphism on obesity-related metabolic disorders in people with excess in body weight. Metabolism. 2007;56(12):1643-8.

637. Vozarova B, Fernández-Real J-M, Knowler WC, Gallart L, Hanson RL, Gruber JD, et al. The interleukin-6 (− 174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. Human Genetics. 2003;112(4):409-13.

638. Woo P, Humphries SE. IL-6 polymorphisms: a useful genetic tool for inflammation research? The Journal of clinical investigation. 2013;123(4):1413-4.

639. Cuenca J, Pérez CA, Aguirre AJ, Schiattino I, Aguillón JC. Genetic polymorphism at position-308 in the promoter region of the tumor necrosis factor (TNF): implications of its allelic distribution on susceptibility or resistance to diseases in the Chilean population. Biological Research. 2001;34(3-4):237-41.

640. Abraham LJ, Kroeger KM. Impact of the-308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. Journal of leukocyte biology. 1999;66(4):562-6.

641. Cuenca J, Cuchacovich M, Perez C, Ferreira L, Aguirre A, Schiattino I, et al. The− 308 polymorphism in the tumour necrosis factor (TNF) gene promoter region and ex vivo lipopolysaccharide-induced TNF expression and cytotoxic activity in Chilean patients with rheumatoid arthritis. Rheumatology. 2003;42(2):308-13.

642. Kroeger KM, Carville KS, Abraham LJ. The− 308 tumor necrosis factor-α promoter polymorphism effects transcription. Molecular immunology. 1997;34(5):391-9.

643. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proceedings of the National Academy of Sciences. 1997;94(7):3195-9.

644. Bouma G, Crusius J, Oudkerk Pool M, Kolkman J, Von Blomberg B, Kostense P, et al. Secretion of tumour necrosis factor α and lymphotoxin α in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scandinavian journal of immunology. 1996;43(4):456-63.

645. Macaluso FS, Maida M, Petta S. Genetic background in nonalcoholic fatty liver disease: A comprehensive review. World Journal of Gastroenterology: WJG. 2015;21(39):11088.

646. Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. Diabetes. 2002;51(7):2306-12.

647. Ukkola O, Ravussin E, Jacobson P, Sjöström L, Bouchard C. Mutations in the adiponectin gene in lean and obese subjects from the Swedish obese subjects cohort. Metabolism. 2003;52(7):881-4.

648. Qi L, Doria A, Manson JE, Meigs JB, Hunter D, Mantzoros CS, et al. Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. Diabetes. 2006;55(5):1512-6.

649. Yang W-S, Chuang L-M. Human genetics of adiponectin in the metabolic syndrome. Journal of molecular medicine. 2006;84(2):112-21.

650. Jang Y, Chae JS, Koh SJ, Hyun YJ, Kim JY, Jeong YJ, et al. The influence of the adiponectin gene on adiponectin concentrations and parameters of metabolic syndrome in non-diabetic Korean women. Clinica Chimica Acta. 2008;391(1-2):85-90.

651. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes. 2007;56(5):1198-209.

652. González-Sánchez JL, Zabena CA, Martínez-Larrad MT, Fernández-Pérez C, Pérez-Barba M, Laakso M, et al. An SNP in the adiponectin gene is associated with decreased serum adiponectin levels and risk for impaired glucose tolerance. Obesity Research. 2005;13(5):807-12.

653. Li X-L, Sui J-Q, Lu L-L, Zhang N-N, Xu X, Dong Q-Y, et al. Gene polymorphisms associated with non-alcoholic fatty liver disease and coronary artery disease: a concise review. Lipids in health and disease. 2016;15(1):53.

654. Liu M, Liu S, Shang M, Liu X, Wang Y, Li Q, et al. Association between ADIPOQ G276T and C11377G polymorphisms and the risk of non-alcoholic fatty liver disease: An updated meta-analysis. Mol Genet Genomic Med. 2019;7(5):e624.

655. Melistas L, Mantzoros CS, Kontogianni M, Antonopoulou S, Ordovas JM, Yiannakouris N. Association of the+ 45T> G and+ 276G> T polymorphisms in the adiponectin gene with insulin resistance in nondiabetic Greek women. European Journal of Endocrinology. 2009;161(6):845-52.

656. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes & Metabolism. 2002;51(2):536-40.

657. Shin M-J, Jang Y, Koh SJ, Chae JS, Kim OY, Lee JE, et al. The association of SNP276G> T at adiponectin gene with circulating adiponectin and insulin resistance in response to mild weight loss. International journal of obesity. 2006;30(12):1702.

658. Uusitupa M. Gene–diet interaction in relation to the prevention of obesity and type 2 diabetes: evidence from the Finnish Diabetes Prevention Study. Nutrition, metabolism and cardiovascular diseases. 2005;15(3):225-33.

659. Ferguson JF, Phillips CM, Tierney AC, Pérez-Martínez P, Defoort C, Helal O, et al. Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. The American journal of clinical nutrition. 2010;91(3):794-801.

660. Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS ONE. 2012;7(8):e43134.

661. Zhao F, Song M, Wang Y, Wang W. Genetic model. Journal of cellular and molecular medicine. 2016;20(4):765.

662. Möhlig M, Boeing H, Spranger J, Osterhoff M, Kroke A, Fisher E, et al. Body mass index and C-174G interleukin-6 promoter polymorphism interact in predicting type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism. 2004;89(4):1885-90.

663. Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis. 2009;41(11):823-8.

664. Humphries S, Luong L, Ogg M, Hawe E, Miller G. The interleukin-6− 174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. European Heart Journal. 2001;22(24):2243-52.

665. Cardellini M, Perego L, D'Adamo M, Marini MA, Procopio C, Hribal ML, et al. C-174G polymorphism in the promoter of the interleukin-6 gene is associated with insulin resistance. Diabetes Care. 2005;28(8):2007-12.

666. Giannitrapani L, Soresi M, Balasus D, Licata A, Montalto G. Genetic association of interleukin-6 polymorphism (-174 G/C) with chronic liver diseases and hepatocellular carcinoma. World J Gastroenterol. 2013;19(16):2449-55.

667. Sookoian SC, González C, Pirola CJ. Meta-analysis on the G-308A Tumor Necrosis Factor α Gene Variant and Phenotypes Associated with the Metabolic Syndrome. Obesity research. 2005;13(12):2122-31.

668. Kurbatova IV, Topchieva LV, Dudanova OP. Gene TNF Polymorphism -308G>A (rs1800629) and Its Relationship with the Efficiency of Ursodeoxycholic Acid Therapy in Patients with Nonalcoholic Stetohepatitis. Bull Exp Biol Med. 2017;164(2):181-5.

669. Kurbatova IV, Topchieva LV, Dudanova OP. Caspase 3, 6, 8, and 9 Gene Expression in Peripheral Blood Leukocytes and Plasma Concentrations of IL-6 and TNF-alpha in Carriers of Different Polymorphic Marker -174G>C Genotypes of IL6 Gene Associated with the Risk of Nonalcoholic Steatohepatitis. Bull Exp Biol Med. 2017;162(3):370-4.

670. Jang Y, Lee JH, Kim OY, Koh SJ, Chae JS, Woo JH, et al. The SNP276G> T polymorphism in the adiponectin (ACDC) gene is more strongly associated with insulin resistance and cardiovascular disease risk than SNP45T> G in nonobese/nondiabetic Korean men independent of abdominal adiposity and circulating plasma adiponectin. Metabolism. 2006;55(1):59-66.

671. Brull D, Montgomery H, Sanders J, Dhamrait S, Luong L, Rumley A, et al. Interleukin-6 gene− 174g> c and− 572g> c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arteriosclerosis, thrombosis, and vascular biology. 2001;21(9):1458-63.

672. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. Journal of applied physiology. 2005;98(4):1154-62.

673. Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. Jama. 2002;288(8):980-7.

674. Curti MLR, Jacob P, Borges MC, Rogero MM, Ferreira SRG. Studies of gene variants related to inflammation, oxidative stress, dyslipidemia, and obesity: implications for a nutrigenetic approach. Journal of Obesity. 2011;2011.

675. Wilson A, De Vries N, Pociot Fd, Di Giovine F, Van der Putte L, Duff G. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. The Journal of experimental medicine. 1993;177(2):557-60.

676. Wilson A, Di Giovine F, Blakemore A, Duff G. Single base polymorphism in the human tumour necrosis factor alpha (TNFα) gene detectable by Ncol restriction of PCR product. Human molecular genetics. 1992;1(5):353-.

677. Aller R, de Luis DA, Izaola O, Gonzalez Sagrado M, Conde R, Alvarez Gago T, et al. G308A polymorphism of TNF-alpha gene is associated with insulin resistance and histological changes in non alcoholic fatty liver disease patients. Annals of Hepatology. 2010;9(4):439-44.

678. Jeng J-S, Tsai J-F, Chuang L-Y, Ho M-S, Lin Z-Y, Hsieh M-Y, et al. Tumor necrosis factor-α 308.2 polymorphism is associated with advanced hepatic fibrosis and higher risk for hepatocellular carcinoma. Neoplasia. 2007;9(11):987-92.

679. Hu Z-w, Luo H-b, Xu Y-m, Guo J-w, Deng X-l, Tong Y-w, et al. Tumor necrosis factor-alpha gene promoter polymorphisms in Chinese patients with nonalcoholic fatty liver diseases. Acta Gastroenterol Belg. 2009;72.

680. Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, et al. Tumor necrosis factor α promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology. 2002;122(2):274-80.

681. Zhou YJ, Li YY, Nie YQ, Yang H, Zhan Q, Huang J, et al. Influence of polygenetic polymorphisms on the susceptibility to non-alcoholic fatty liver disease of Chinese people. J Gastroenterol Hepatol. 2010;25(4):772-7.

682. Chen X, Xun K, Chen L, Wang Y. TNF-α, a potent lipid metabolism regulator. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. 2009;27(7):407-16.

683. Joffe YT, Collins M, Goedecke JH. The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids. Nutrients. 2013;5(5):1672-705.

684. Madej A, Okopień B, Kowalski J, Zieliński M, Wysocki J, Szyguła B, et al. Levels of tumor necrosis factor alpha in serum of patients with hyperlipoproteinemia IIB before and after micronized fenofibrate therapy. Polskie Archiwum Medycyny Wewnetrznej. 1998;99(4):308-13.

685. Rönnelid J, Wick MC, Lampa J, Lindblad S, Nordmark B, Klareskog L, et al. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Annals of the rheumatic diseases. 2005;64(12):1744-9.

686. Chandrasekar B, Fernandes G. Decreased proinflammatory cytokines and increased antioxidant enzyme gene expression by ω-3 lipids in murine lupus nephritis. Biochemical and biophysical research communications. 1994;200(2):893-8.

687. Pérez-Matute P, Pérez-Echarri N, Martínez JA, Marti A, Moreno-Aliaga MJ. Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-α. British Journal of Nutrition. 2007;97(2):389-98.

688. Fontaine-Bisson B, Wolever TM, Chiasson J-L, Rabasa-Lhoret R, Maheux P, Josse RG, et al. Genetic polymorphisms of tumor necrosis factor-α modify the association between dietary polyunsaturated fatty acids and fasting HDL-cholesterol and apo AI concentrations. The American journal of clinical nutrition. 2007;86(3):768-74.

689. Joffe YT, van der Merwe L, Carstens M, Collins M, Jennings C, Levitt NS, et al. Tumor necrosis factor-α gene-308 G/A polymorphism modulates the relationship between dietary fat intake, serum lipids, and obesity risk in black south African women. The Journal of nutrition. 2010;140(5):901-7.

690. Joffe YT, van der Merwe L, Collins M, Carstens M, Evans J, Lambert EV, et al. The-308 G/A polymorphism of the tumour necrosis factor-α gene modifies the association between saturated fat intake and serum total cholesterol levels in white South African women. Genes & nutrition. 2011;6(4):353-9.

691. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, et al. Diet and overall survival in elderly people. Bmj. 1995;311(7018):1457-60.

692. Lee CC, You N-cY, Song Y, Hsu Y-H, Manson J, Nathan L, et al. Relation of genetic variation in the gene coding for C-reactive protein with its plasma protein concentrations: findings from the Women's Health Initiative Observational Cohort. Clinical chemistry. 2009;55(2):351-60.

693. Illig T, Bongardt F, Schopfer A, Muller-Scholze S, Rathmann W, Koenig W, et al. Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. The Journal of Clinical Endocrinology Metabolism. 2004;89(10):5053-8.

694. Li H-J, Li C-P, Zhang C, Zhong X-L, Shi L. Association of Adiponectin gene polymorphisms and nonalcoholic fatty liver disease. International journal of clinical experimental medicine. 2015;8(9):16676.

695. Wei Z, Li-Qun Z, Xiao-Ling H, Jian Q, Guo-Yue Y. Association of adiponectin gene polymorphisms and additional gene-gene interaction with nonalcoholic fatty liver disease in the Chinese Han population. Hepatol Int. 2016;10(3):511-7.

696. Musso G, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. Hepatology. 2008;47(4):1167-77.

697. Kang ES, Park SY, Kim HJ, Ahn CW, Nam M, Cha BS, et al. The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with type 2 diabetes. Diabetes Care. 2005;28(5):1139-44.

698. Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, et al. The adiponectin gene SNP+ 276G> T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age≤ 50 years). Journal of molecular medicine. 2005;83(9):711-9.

699. Maiorino MI, Bellastella G, Petrizzo M, Scappaticcio L, Giugliano D, Esposito K. Mediterranean diet cools down the inflammatory milieu in type 2 diabetes: the MÉDITA randomized controlled trial. Endocrine. 2016;54(3):634-41.

700. Lasa A, Miranda J, Bulló M, Casas R, Salas-Salvadó J, Larretxi I, et al. Comparative effect of two Mediterranean diets versus a low-fat diet on glycaemic control in individuals with type 2 diabetes. European journal of clinical nutrition. 2014;68(7):767-72.

701. Mayr HL, Tierney AC, Kucianski T, Thomas CJ, Itsiopoulos C. Australian patients with coronary heart disease achieve high adherence to 6-month Mediterranean diet intervention: preliminary results of the AUSMED Heart Trial. Nutrition. 2019;61:21-31.

702. Davis C, Hodgson J, Bryan J, Garg M, Woodman R, Murphy K. Older Australians can achieve high adherence to the Mediterranean diet during a 6 month randomised intervention; results from the Medley Study. Nutrients. 2017;9(6):534.

703. Zazpe I, Sanchez-Tainta A, Estruch R, Lamuela-Raventos RM, Schröder H, Salas-Salvado J, et al. A large randomized individual and group intervention conducted by registered dietitians increased adherence to Mediterranean-type diets: the PREDIMED study. Journal of the American Dietetic Association. 2008;108(7):1134-44.

704. Peña-Romero AC, Navas-Carrillo D, Marín F, Orenes-Piñero E. The future of nutrition: nutrigenomics and nutrigenetics in obesity and cardiovascular diseases. Critical reviews in food science and nutrition. 2018;58(17):3030-41.

705. Ferguson JF, Allayee H, Gerszten RE, Ideraabdullah F, Kris-Etherton PM, Ordovás JM, et al. Nutrigenomics, the microbiome, and gene-environment interactions: new directions in cardiovascular disease research, prevention, and treatment: a scientific statement from the American Heart Association. Circulation: Cardiovascular Genetics. 2016;9(3):291-313.

706. Vickers NJ. Animal communication: when i'm calling you, will you answer too? Current biology. 2017;27(14):R713-R5.

707. Vilarinho S, Mistry PK. Exome sequencing in clinical hepatology. Hepatology. 2019;70(6):2185-92.

708. Glasziou P, Irwig L, Bain C, Colditz G. How to use the evidence: assessment and application of scientific evidence. Canberra: National Health & Medical Research Council; 2000. p. 85.

709. Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. Journal of reproductive immunology. 2005;66(2):175-91.

710. Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Digestive and Liver Disease. 2008;40(5):371-8.

711. Lansang MC, Williams GH, Carroll JS. Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. American journal of hypertension. 2001;14(1):51-3.

712. Salgado ALFdA, Carvalho Ld, Oliveira AC, Santos VNd, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arquivos de gastroenterologia. 2010;47(2):165-9.

713. Poslusna K, Ruprich J, de Vries JH, Jakubikova M, van't Veer P. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. British Journal of Nutrition. 2009;101(S2):S73-S85.

714. Poppitt S, Swann D, Black A, Prentice A. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. International journal of obesity. 1998;22(4):303-11.

715. Jayakumar S, Loomba R. Emerging role of the gut microbiome in the progression of nonalcoholic fatty liver disease and potential therapeutic implications. Alimentary Pharmacology & Therapeutics. 2019;50(2):144-58.

716. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology. 2016;63(3):764-75.

717. Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013;58(1):120-7.

Appendices

Appendix 1 Published manuscript and supplementary materials published in association to the article

Nutrition in Clinical Care

Effect of dietary intervention, with or without co-interventions, on inflammatory markers in patients with nonalcoholic fatty liver disease: a systematic literature review

Anjana J. Reddy, Elena S. George, Stuart K. Roberts, and Audrey C. Tierney

Context: *Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of liver disorders, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), with inflammation acting as a key driver in its pathogenesis and progression. Diet has the potential to mediate the release of inflammatory markers; however, little is known about the effects of various diets.* ***Objective:*** *This systematic review aimed to evaluate the effect of dietary interventions on cytokines and adipokines in patients with NAFLD.* ***Data Sources:*** *The electronic databases MEDLINE, EMBASE, CINAHL, and Cochrane Library were searched for clinical trials investigating dietary interventions, with or without supplementation, on cytokines and adipokines in NAFLD patients.* ***Data Extraction:*** *Basic characteristics of populations, dietary intervention protocol, cytokines, and adipokines were extracted for each study. Quality of evidence was assessed using the American Dietetic Association criteria.* ***Data Analysis:*** *Nineteen studies with a total of 874 participants were included. The most frequently reported inflammatory outcomes were C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, and leptin. Hypocaloric, isocaloric, or low-fat diets significantly ($P < 0.05$) lowered levels of CRP, TNF-α, and adiponectin. The addition of nutraceutical or pharmacological supplementation to dietary interventions appeared to elicit additional benefits for all of the most frequently reported inflammatory markers.* ***Conclusions:*** *Hypo- or isocaloric diets alone, or with co-interventions that included a nutraceutical or pharmacological supplementation, appear to improve the inflammatory profile in patients with NAFLD. Thus, anti-inflammatory diets may have the potential to improve underlying chronic inflammation that underpins the pathophysiological mechanisms of NAFLD. In the absence of any known liver-sensitive markers, the use of cytokines and adipokines as a surrogate marker of liver disease should be further investigated in well-controlled trials.*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in developed countries,[1] affecting at least 25% of adults.[2] Rates of NAFLD parallel the obesity epidemic and are present in up to 80% of obese individuals and 75% of people with type 2 diabetes.[3,4]

Affiliation: *A.J. Reddy*, *E.S. George*, and *A.C. Tierney* are with the Department of Rehabilitation, Nutrition and Sport, La Trobe University, Bundoora, Victoria, Australia. *E.S. George* is with the Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, Victoria, Australia. *E.S. George* and *A.C. Tierney* are with the Department of Nutrition, Alfred Health, Prahran, Victoria, Australia. *S.K. Roberts* is with the Department of Gastroenterology, Alfred Health, Prahran, Victoria, Australia. *A.C. Tierney* is with the School of Allied Health, University of Limerick, Limerick, Ireland.

Correspondence: *A.J. Reddy*, La Trobe University, Room 315, Health Sciences Building 3, 1 Kingsbury Dr, Bundoora, Melbourne, VIC 3086, Australia. E-mail: A.Reddy@latrobe.edu.au.

Key words: adipokines, cytokines, dietary intervention, inflammatory markers, nonalcoholic fatty liver disease.

© The Author(s) 2019. Published by Oxford University Press on behalf of the International Life Sciences Institute.
All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

doi: 10.1093/nutrit/nuz029

Downloaded from https://academic.oup.com/nutritionreview/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

Although the pathogenesis of NAFLD is not well understood, Tilg and Moschen have proposed a "multiple parallel hit" hypothesis, suggesting that inflammatory mediators derived from various tissues, specifically adipose tissue and the gut, play a central role in the cascade of inflammation and fibrosis.[5] Adipose tissue itself can produce and secrete pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), as well as adipokines and adiponectin and leptin, which are both implicated in the progression of insulin resistance (IR) and metabolic dysregulation in NAFLD.[6,7] In contrast with leptin, adiponectin secretion is often diminished in obesity and acts to increase insulin sensitivity.[6] In response to the secretion of cytokines, extrahepatic production of the acute-phase protein high-sensitivity C-reactive protein (hs-CRP) exacerbates a pro-inflammatory milieu and drives further hepatic and cardiometabolic damage.[8,9]

There is currently no proven, safe, and effective pharmacotherapy for the treatment of NAFLD.[10] Current recommendations emphasize weight loss, which may be achieved through management of lifestyle, including diet.[11] Dietary intakes of individuals with NAFLD have been reported to be high in saturated fat, refined carbohydrates, fructose, and cholesterol and low in antioxidants and omega-3 fatty acids.[12] These diets are known to exacerbate inflammatory cytokine and adipokine production, release free fatty acids (FFAs), stimulate oxidative stress, and influence disease progression in metabolic diseases.[8] Furthermore, overfeeding can cause impaired energy homeostasis, appetite dysregulation, and weight fluctuation, which is regulated by the pro-inflammatory cytokine, leptin.[13] One of the main physiological roles of leptin is to prevent lipid accumulation in nonadipose sites, including the liver.[14] Although leptin is not commonly reported in existing studies, patients with NAFLD tend to have increased serum leptin concentrations.[15]

Low-fat diets, although well-researched in chronic disease management, show variable results for the effects on inflammatory markers and seem to be dependent on weight loss.[16] Hypocaloric diets typically provide an energy deficit of 500–1000 kcal/day and are aimed at inducing a total body weight loss of approximately 5%–10%,[17] which may ameliorate hepatic and metabolic outcomes via a reduction in adiposity and improvement of glucose and lipid metabolism.[18]

However, weight loss can be difficult to achieve and maintain, and thus isocaloric diets that aim for energy balance focus on dietary components that are anti-inflammatory in nature.[19] This includes the Mediterranean diet, which is predominantly plant-based, high in fiber, high in monounsaturated and polyunsaturated fats, and has anti-inflammatory properties[11,12] and thus may alleviate hepatic and cardiometabolic stress irrespective of weight loss.[20–24]

Alternative therapies, including nutraceuticals (ie, substances derived from biologically active isolated nutrients or functional foods) are being increasingly considered in the treatment of NAFLD.[25–27] Presently, there is not enough substantial evidence to make any recommendations for the use of nutraceutical agents in the management of NAFLD.

Despite the number of trials that have assessed varying diet and supplementation approaches, there is currently no consensus regarding the optimal dietary intervention(s) to improve the inflammatory milieu within the liver that is responsible for hepatocyte injury and fibrosis in individuals with NAFLD. Hence, the present systematic review aims to assess the current literature and to determine the effect of dietary interventions on cytokines and adipokines in adults diagnosed with NAFLD.

METHOD

This systematic review adheres to the relevant criteria of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (see Appendix S1 in the Supporting Information online)[28] and the Cochrane Handbook for Systematic Reviews of Interventions.[29] The review was registered in PROSPERO, the international prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO; registration no.: CRD42017055921).

Search strategy

A search for all relevant articles was performed using the electronic databases MEDLINE Ovid (1946–present), EMBASE Ovid (1947–present), CINAHL (EBSCO), and the Cochrane Library (Wiley Online Library). The last search was run on January 15, 2018. English language limits were applied. The search strategy used combinations of the terms "nonalcoholic fatty liver disease," "NAFLD," "nonalcoholic steatohepatitis (NASH)," "cirrhosis," "diet," and "nutrition" as both medical subject headings (MeSH) and subject headings specific to each database and keywords or free-text words and included a wide range of derivations to ensure an extensive search was performed (see Appendix S2 in the Supporting Information online). The search was not limited to specific outcomes to ensure all relevant literature investigating cytokines and adipokines was captured. Citation tracking and hand-searching of the reference lists of relevant reviews and articles that were retrieved in searches were also undertaken.

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Conference abstracts and reports were also screened, and the full articles of potentially eligible studies were retrieved.

Eligibility criteria

The inclusion and exclusion criteria were developed using the Patient, Intervention, Comparators, Outcome, and Study Design (PICOS)[30] method (Table 1).

References were imported into a bibliographic database to automatically exclude duplicates (EndNote X7.4). References were screened in duplicate by 2 researchers by title and abstract, and full publications of potentially eligible references were obtained.

Quality assessment and data extraction

Once eligible studies were identified, 2 independent researchers assessed the methodological quality of each using the American Dietetic Association Quality Criteria Checklist for Primary Research.[31] The criteria checklist for validity assessment contained 10 questions. A study was considered negative (−) if >6 validity questions were answered "no"; a study was considered unclear (±) if 4 specific validity questions were answered "yes"; and a study was considered positive (+) if most validity questions were answered "yes."

The process of extracting data from eligible articles was then completed independently by 1 researcher, after which a second reviewer cross-checked all extracted data. When articles contained insufficient information to perform quality assessment or extract relevant data, the corresponding author was contacted for further information. This occurred for 5 articles.[32–36] Two authors responded.[35,36] Disagreements regarding eligibility, quality assessment, and data extraction were resolved through discussion and consensus.

Data analysis

A meta-analysis was not undertaken due to the heterogeneity of the dietary interventions, study designs, and participants within the included studies, as well as inconsistent control and experimental intervention groups, including co-interventions. Due to this variability, researchers were unable to group dietary interventions for analysis. Where numerical values for inflammatory markers were presented in different units (eg, mmol/L vs mg/dL), measures were converted into the same unit to allow comparisons to be made. The difference in means and level of significance were extracted from each study, and change was calculated as a percentage.

RESULTS

A total of 3855 articles were retrieved from the database search, and after duplicates were removed 2993 remained. Following a review of titles and abstracts, 79 were deemed potentially eligible. Full-text articles were examined, and 20 fulfilled the inclusion criteria. One article was excluded because it contained no result tables or figures with numerical values and no response was obtained after contacting the authors.[37] Nineteen studies were therefore included. Reference lists of all eligible studies and relevant reviews were checked for potential inclusions; however, no additional articles were retrieved. The study selection process is summarized in Figure 1.

All 19 included studies were randomized controlled trials (RCTs): 3 were nonblinded[33,38,39]; 2 were single-blinded[34,40]; 3 were double-blinded[32,36,41]; 7 were double-blind, placebo-controlled[42–48]; 3 were open-label, parallel-arm[35,49,50]; and 1 study was a prospective, single-blinded, random-order, controlled dietary feeding study.[51]

Study characteristics and participants

Studies included in this review were published between 2003 and 2018; there were a total of 874 participants with NAFLD, and the length of interventions ranged from 2 weeks to 12 months. Of the overall sample, 488 (56%) were males and 386 (44%) were females. The age of participants ranged from 36 to 65 years, and body mass index (BMI) ranged 23–35 kg/m^2. Three of the 19 studies used the gold-standard liver biopsy (Bx) to diagnose NAFLD,[34,38,45] 3 used magnetic resonance spectroscopy (^{1}H-MRS),[35,40,51] 2 used abdominal ultrasound alone,[32,42] 1 used Fibroscan alone,[50] 2 used a combination of Fibroscan and liver enzymes,[46,47] 1 used a combination of ultrasound and Fibroscan,[49] and 7 used a combination of ultrasound and liver enzymes.[33,36,39,41,43,44,48] Characteristics of each study, patient population, and study design are presented in Table 2.d[32–36,38–45,47–51]

Intervention characteristics

Of the 19 studies included in this review, 2 compared a hypocaloric diet with a hypocaloric diet plus a co-intervention (a cholesterol absorption inhibitor and an oral hypoglycemic agent).[32,40] One study compared a hypocaloric diet with a Dietary Approaches to Stop Hypertension (DASH) diet,[41] and 1 compared an isocaloric diet with an isocaloric diet plus the addition of Corinthian currants.[49] Two studies compared an energy-balanced diet with an energy-balanced diet with the addition of a synbiotic supplement,[44,46] and 4 studies compared an energy-balanced diet with an energy-balanced diet plus supplementation (prebiotic,

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

Table 1 **PICOS criteria for inclusion and exclusion of studies**

PICOS	Inclusion/exclusion criteria[a]	Data extracted
Population	Inclusion: Adults aged ≥18 y, diagnosed with NAFLD using >1 of the following diagnostic criteria: 1) histological examination of biopsies; 2) magnetic resonance imaging and/or magnetic resonance spectroscopy; 3) computed tomography; 4) ultrasound; and 5) blood concentrations of liver enzymes alanine aminotransferase and/or aspartate aminotransferase Exclusion: Any animal, pediatric, or pregnancy studies	Location (country), method of NAFLD diagnosis, no. of participants, age, sex, body mass index
Intervention	Inclusion: Studies that compared a dietary intervention with an alternative diet or control. Studies where supplementation was provided alongside a dietary intervention, as long as there was an independent dietary intervention group (eg, supplementation plus dietary intervention vs dietary intervention alone) Interventions that included a dietary intervention alongside a co-intervention such as physical activity, behavior training, or other lifestyle interventions were eligible if the control or other diet arm was stand-alone (ie, dietary intervention only). Studies that suggested physical activity recommendations alongside both dietary intervention and control groups were included if these recommendations were consistent among groups and not a primary outcome Exclusion: Studies that intervened only with supplements or pharmacological drugs or investigated only postprandial effects of a dietary or meal intervention	Intervention length, type of dietary intervention, dietary intervention protocol The addition of supplementation or co-intervention
Comparators	Inclusion: Control group or stand-alone diet Exclusion: Studies without a comparator group	Intervention length, type of dietary intervention, dietary intervention protocol
Outcomes	Inclusion: Studies that reported outcomes of inflammatory cytokines and/or adipokines Exclusion: Studies that did not present results as numerical values for inflammatory cytokines and/or adipokines	Type of inflammatory marker. Pre- and post- intervention results of each inflammatory marker
Study design	Inclusion: The current review included only randomized controlled trials. Publications were eligible if they were published in peer-reviewed scientific journals, written in English language or had English versions of foreign language studies available Exclusion: Reviews, cohort studies, cross-sectional studies, case-control studies, conference abstracts, editorials, letters, and reviews. Non-English language only papers	Type of study design Level of evidence of each study, as determined using the NHMRC Evidence Hierarchy Methodological quality of each study using the American Dietetic Association Quality Criteria Checklist for Primary Research[31]

[a]Where 2 reports relate to the same patient group, the most complete report was included to avoid duplication of patient numbers.
Abbreviations: NAFLD, nonalcoholic fatty liver disease; NHMRC, National Health and Medical Research Council.

probiotic, ginger, green coffee bean extract [GCBE], or flaxseed).[43,47,48,50] Four studies used a low-fat diet (LFD) intervention (American Diabetes Association guide for weight-management diet,[42] National Cholesterol Education Program [NCEP] Adult Treatment Panel III therapeutic lifestyle-change diet,[45] and Step One American Heart Association [AHA] Diet)[34,39] compared with the same LFD plus supplementation (soy isoflavone,[42] L-carnitine,[45] vitamin E,[34] and n-3 polyunsaturated fatty acid [PUFA]).[39] One study compared an LFD with a high-fat diet (HFD),[51] another study compared a plant protein isocaloric diet with an animal protein isocaloric diet,[35] and another compared a Mediterranean diet with an identical diet plus olive oil enriched with n-3 PUFA.[36] A trial with 3 intervention arms compared a low-calorie diet, a low-calorie and low-carbohydrate diet, and a soy-containing, low-calorie, low-carbohydrate diet.[33]

Protocols for the dietary interventions were diverse; the nutrient composition and caloric intake targets, major food sources, and physical activity (PA) recommendations are detailed in Table 3.[32–36,38–51] Definitions for the calorie-restricted diets ranged from unspecified[40] to a 250-kcal-per-day deficit to 700-kcal-per-day deficit[41]; in most cases, caloric requirements were calculated on an individual basis and were dependent on baseline BMI. The energy-balanced diet and PA recommendations implemented in 6 studies[43,44,46–48,50] were according to Clinical Guidelines for the Study of Obesity.[52] The Mediterranean diet protocol was unspecified.[36]

Inflammatory markers

Cytokines.

The most commonly analyzed cytokines in the included studies were hs-CRP, TNF-α, and IL-6, which were reported in 12,[33,38,40,41,44–51] 11,[35,39,40,42,44–50] and 6 studies, respectively.[35,38,40,42,49,51] Data extracted for intervention effects of cytokines within each study are presented in Table 4.[33,35,38–42,44–51]

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

Table 2 **Characteristics of studies included for the systematic review investigating the effects of randomized controlled dietary intervention(s) on inflammatory markers in adults with nonalcoholic fatty liver disease**

Reference	Country	NAFLD diagnostic method	Sample (n), M/F	Study type/ NHMRC LOE and Quality Ax	Diet of Interest	2° diet of interest	3° diet of interest	Intervention length	Inflammatory biomarkers measured
Amanat et al (2017)[42]	Iran	US	Enrolled (n=82), analyzed (n=78), 61/21	RCT/Level II Positive	Weight-management diet plus placebo	Weight-management diet plus soy isoflavone supplement	—	8 wk	TNF-α, IL-6
Baldry et al (2017)[38]	United Kingdom	Liver Bx	Total (n=54), 44/10	RCT/Level II Positive	Very-low-energy diet in the form of standard pre-bariatric surgery food-based diet	Very-low-energy diet (VLED) in the form of a meal-replacement plan	—	2 wk	hs-CRP, IL-6, fetuin-A
Behrouz et al (2017)[43]	Iran	US and ALT (>1.5 x upper limit of normal)	Total (n=89), 63/26	RCT/ Level II Positive	Energy-balanced diet plus prebiotic and probiotic placebo	Energy-balanced diet plus probiotic supplement and prebiotic placebo	Energy-balanced diet plus prebiotic supplement and probiotic placebo	12 wk	Adiponectin, leptin
Chan et al (2010)[40]	Australia	MR-S (IHTG %)	Total obese and T2DM (n=25), 15/10	RCT/Level II Positive	16-wk hypocaloric, low-fat diet, followed by 6-wk isocaloric diet plus placebo supplement consumed for 22 wk	16-wk hypocaloric, low-fat diet, followed by 6-wk isocaloric diet plus 10 mg/d ezetimibe consumed for 22 wk	—	22 wk	Adiponectin, hs-CRP, TNF-α, IL-6, RBP-4, fetuin-A
Eslamparast et al (2014)[44]	Iran	US and ALT (>60 U/L)	Total (n=52), 25/27	RCT/Level II Positive	Energy-balanced diet plus placebo	Energy-balanced diet plus synbiotic supplement	—	28 wk	hs-CRP, TNF-α, NF-κB
Garinis et al (2010)[32]	Italy	US	Total (n=45), 7/38	RCT/Level II Positive	Hypocaloric diet	Hypocaloric diet plus metformin 1000 mg/d	—	6 mo	Adiponectin
Kaliora et al (2016)[49]	Greece	US	Total (n=55), 23/32	RCT/Level II Positive	Isocaloric diet	Isocaloric diet plus Corinthian currants	—	24 wk	hs-CRP, TNF-α, IL-6, leptin, visfatin
Kani et al (2014)[33]	Iran	US, ALT, and AST (M >30 U/L, F >20 U/L)	Total (n=45), 21/24	RCT/Level II Positive	Low-calorie diet	Low-calorie, low-carbohydrate diet	Low-calorie, low-carbohydrate soy diet	8 wk	hs-CRP
Kugelmas et al (2003)[34]	United States	Liver Bx	Total (n=16), 7/9	RCT/Level II Positive	Step One American Heart Association diet	Step One American Heart Association diet plus vitamin E 800 IU p/d	—	12 wk	TNF-α, IL-6, IL-8

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 2 **Continued**

Reference	Country	NAFLD diagnostic method	Sample (n), M/F	Study type/ NHMRC LOE and Quality Ax	Diet of Interest	2° diet of interest	3° diet of interest	Intervention length	Inflammatory biomarkers measured
Malaguarnera et al (2010)[45]	Italy	Liver Bx	Total (n=74), 40/34	RCT/Level II Positive	National Cholesterol Education Program diet plus placebo	National Cholesterol Education Program diet plus L-carnitine	—	24 wk	hs-CRP, TNF-α
Marina et al (2014)[51]	United States	MR-S	Total obese sample (n=13), 10/3	Random order, comparative study with concurrent controls/ Level III-2 Positive	Low-fat diet	High-fat diet	—	4 wk	Adiponectin, leptin, hs-CRP, IL-6, IL-10, IL-12, IFN-γ
Markova et al (2016)[35]	Germany	MR-S	Total (n=37), 24/13	RCT/Level II Positive	Plant protein isocaloric diet	Animal protein isocaloric diet	—	6 wk	Adiponectin, TNF-α, IL-4, IL-6, IL-8, IL-18, MCP-1
Mofidi et al (2017)[46]	Iran	Fibroscan and ALT (>60 U/L)	Total (n=42), 23/19	RCT/Level II Positive	Energy-balanced diet plus placebo	Energy-balanced diet plus synbiotic supplement	—	28 wk	hs-CRP, TNF-α, NF-κB
Rahimlou et al (2016)[47]	Iran	Fibroscan and ALT (>1.5 x upper limit of normal)	Total (n=44), 20/24	RCT/Level II Positive	Energy-balanced diet plus placebo	Energy-balanced diet plus ginger supplement	—	12 wk	hs-CRP, TNF-α
Razavi Zade et al (2016)[41]	Iran	US and ALT (M >30 U/L, F >19 U/L)	Total (n=60), 30/30	RCT/Level II Positive	Hypocaloric diet	Dietary Approaches to Stop Hypertension diet	—	8 wk	hs-CRP
Shahmohammadi et al (2017)[48]	Iran	US and ALT (M >30 U/L, F >19 U/L)	Total (n=44), 22/22	RCT/Level II Positive	Energy-balanced diet plus placebo	Energy-balanced diet plus green coffee bean extract supplement	—	8 wk	hs-CRP, TNF-α
Sofi et al (2010)[36]	Italy	US and ALT (M >30 U/L, F >20 U/L)	Total (n=11), 9/2	RCT/Level II Positive	Mediterranean diet	Mediterranean diet + olive oil enriched with n-3 PUFA	—	12 mo	Adiponectin
Spadaro et al (2008)[39]	Italy	US and ALT (M >30 U/L, F >20 U/L)	Total (n=36), 19/17	RCT/Level II Positive	American Heart Association diet plus placebo	American Heart Association diet plus n-3 PUFA capsule	—	6 mo	TNF-α
Yari et al (2016)[50]	Iran	Fibroscan	Total (n=50), 25/25	RCT/Level II Positive	Energy-balanced diet	Energy-balanced diet plus flaxseed supplement	—	12 wk	hs-CRP, TNF-α

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Ax, assessment; Bx, biopsy; F, female; hs-CRP, high sensitivity C-reactive protein; IFN-γ, interferon gamma; IHTG%, intrahepatic triglyceride percent; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IL-12, interleukin 12; IL-18, interleukin 18; LOE, level of evidence; M, male; MCP-1, monocyte chemoattractant protein 1; MR-S, magnetic resonance–spectroscopy; n-3, omega 3; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor kappa B; NHMRC, National Health and Medical Research Council; PUFA, polyunsaturated fatty acid; RPB-4, retinol binding protein 4; RCT, randomized controlled trial; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-alpha; US, ultrasound.

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

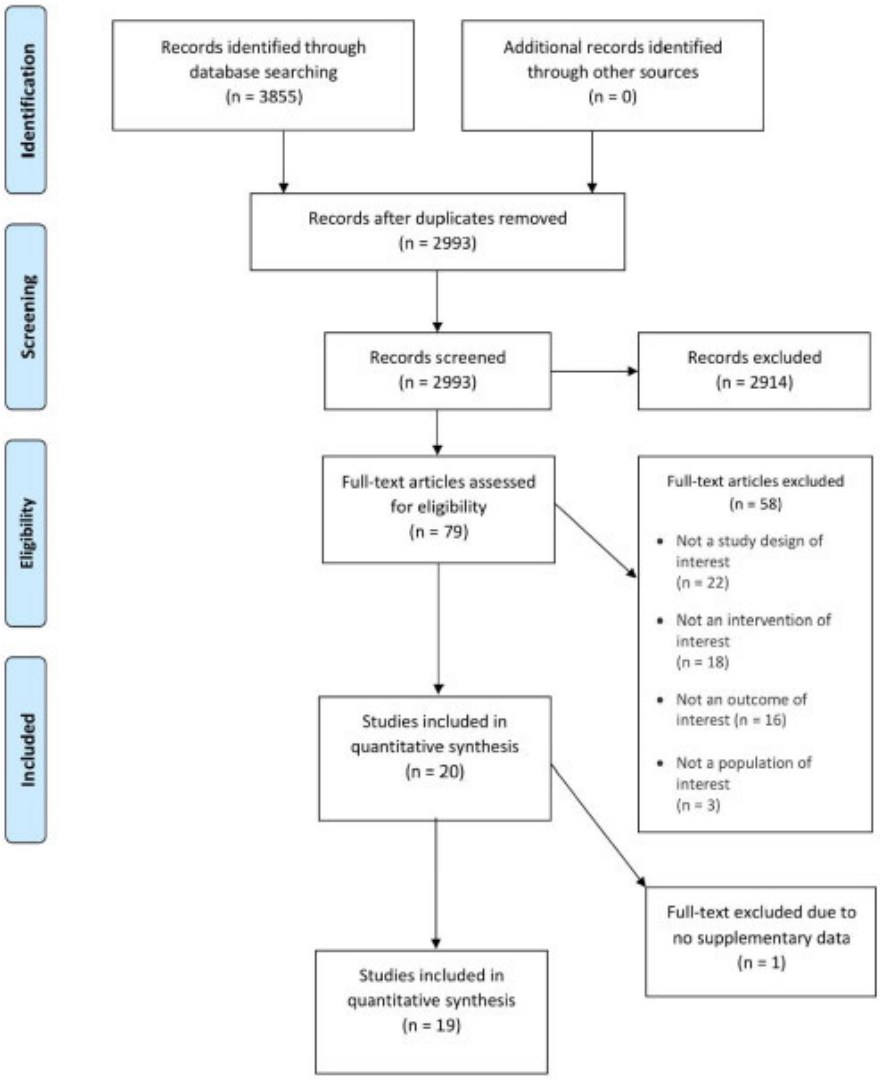


Figure 1 **PRISMA flow chart for study selection**

High-sensitivity C-reactive protein. Of the 12 studies that evaluated hs-CRP, 11 studies reported significant ($P < 0.05$) improvements from pre- to post-intervention, and 1 study reported nonsignificant ($P > 0.05$) improvements (Table 4).[33,35,38–42,44–51] Kaliora et al[49] conducted a 24-week RCT that found that participants who received isocaloric dietary advice alone and participants who received isocaloric dietary advice with an additional 35 g of Corinthian currents both significantly improved hs-CRP (P=0.023 and 0.002, respectively). No significant differences were seen between treatment groups (P=0.748). After an 8-week intervention comparing a hypocaloric diet with a DASH diet, there was a significant reduction in hs-CRP for the DASH diet group only (P=0.08 and 0.004, respectively).[41] Another 8-week intervention saw a reduction in hs-CRP following a low-calorie, low-carbohydrate, soy-containing diet (P=0.01).[33] Both a food-based and a meal replacement very-low-energy diet (VLED) significantly reduced hs-CRP (P=0.007 and 0.004, respectively).[38] Of the studies intervening with diet plus supplementation, Chan et al[40] reported significant improvements following a

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 3 **Dietary intervention protocol data extracted from each study**

Reference	Diet label	Nutrient composition targets	Caloric intake recommendations	Main food sources	Physical activity recommendations
Amanat et al (2017)[42]	American Diabetes Association Guidelines or "Weight-management diet"	<25%–30% of total energy as fat (<7% as SFAs, 20% as MUFAs, and 10% as PUFAs), 15% as protein, 50%–60% as carbohydrate, <200 mg/d as dietary cholesterol, and 20–30 g fiber/d	Energy intake goal to achieve a 500–1000 kcal/d energy deficit	A variety of fruits, vegetables, grains, low-fat or nonfat dairy products, fish, legumes, poultry, and lean meats. Limit foods high in saturated fat, trans fatty acids, and cholesterol; substitute unsaturated fat from vegetables, fish, legumes, and nuts. Emphasize a diet rich in fruits, vegetables, and low-fat dairy products. Limit salt to 6 g/d (2400 mg sodium) by choosing foods low in salt. Limit alcohol intake to <2 drinks per day (men) and 1 drink per day (women)	Initial physical activity recommendations of 30–45 min of moderate aerobic activity, 3–5 d per week, when possible. Greater activity levels of at least 1 h per day of moderate (walking) or 30 min per day of vigorous (jogging) activity to achieve successful long-term weight loss
Baldry et al (2017)[38]	Very-low-energy diet; pre-bariatric surgery food-based diet	ns	800 kcal/d	Standard pre-bariatric surgery food-based diet using LighterLife Nutritional supplements	ns
	Very-low-energy diet; pre-bariatric surgery meal replacement plan	ns	800 kcal/d	Standard pre-bariatric surgery meal replacement plan using LighterLife Nutritional supplements	ns
Behrouz et al (2017)[43]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week
Chan et al (2010)[40]	Hypocaloric, LF	ns	ns	ns	ns
	Isocaloric	ns	ns	ns	ns
Eslamparast et al (2014)[44]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 3 **Continued**

Reference	Diet label	Nutrient composition targets	Caloric intake recommendations	Main food sources	Physical activity recommendations
Garinis et al (2010)[32]	Hypocaloric	ns	1300 kcal consumed per day	ns	ns
Kaliora et al (2016)[49]	Isocaloric	30% of the total energy as fat (<10% as SFAs, ~10% as MUFAs, and ~10% as PUFAs), 20% as protein, 50% as carbohydrate, 300 mg/d as dietary cholesterol, and 20–30 g fiber/d	Daily energy needs were determined according to the basic metabolic rate equation of Harris–Benedict and sedentary lifestyle	Participants in both diet groups received the same dietary counseling. The Current arm incorporated in their daily diet the consumption of 36 g of Corinthian currants equal to 2 fruit servings replacing snacks of like nutritional value (low-fat yogurt, mini crackers, or bread with low-fat cheese)	Aim of nutritional counseling was a weight loss of ≈5% of the initial BW within 6 mo
Kani et al (2014)[33]	Low-calorie	55% of calories were supplied by carbohydrates, 30% by fats, and 15% by proteins	Calorie restriction was considered according to participant's BMI category. A 200-calorie reduction was considered for overweight individuals and up to a 500-calorie reduction for obese participants	ns	Recommended that all participants engage in moderate physical activity for 30 min a day
	Low-calorie, low-carbohydrate	45% of the calories were supplied by carbohydrates, 35% by fats, and 20% by proteins		ns	
	Low-calorie, low-carbohydrate soy containing	Composition of the macronutrients was similar to the low-calorie, low-carbohydrate group except in this diet 30 g of soy nut was incorporated instead of 30 g of red meat		Soy nut was provided in suitable amounts in a separated box with a small glass showing 30 g	
Kugelmas et al (2003)[34]	AHA	ns	ns	ns	ns
Malaguarnera et al (2010)[45]	NCEP	50%–60% of total energy as carbohydrates, 15% as protein, and 25%–35% as fat	Patients in both the groups were given the same 1600-calorie diet	ns	Both the groups were prescribed an exercise plan and a 30-min home-based whole-body stretching routine to perform 3 times per week
Marina et al (2014)[51]	Low-fat	20% total energy as fat (8% saturated fat) and 62% as carbohydrates	Caloric needs were estimated using the average of the Mifflin-St. Jeor and Harris-Benedict equations, adjusted for physical activity	Major sources of fats in both diets included butter and high oleic safflower oil. Vegetable content was matched. Because fructose was limited on the HFD due to the low carbohydrate content, fructose was limited in both diets to <30 g/d based on a 2000 kcal per day diet	Participants were instructed to maintain regular physical activity and to eat all of the food provided, not to eat any nonstudy food, and to report any deviations from the diet
	High-fat	55% total energy as fat (25% saturated fat) and 27% as carbohydrates			

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 3 **Continued**

Reference	Diet label	Nutrient composition targets	Caloric intake recommendations	Main food sources	Physical activity recommendations
Markova et al (2016)[35]	Plant protein isocaloric	30% of total energy as protein, 40% as carbohydrates, and 30% as fat (10% SFA, 10% MUFA, 10% PUFA)	Energy intake of participants was estimated using reports on daily intake and physical activity and resting energy expenditure measured by indirect calorimetry	Foods enriched with pea proteins specially developed for this study (eg, noodles, a pea protein drink, a mash potato, a pea protein bread, and cookies)	ns
	Animal protein isocaloric	30% of total energy as protein, 40% as carbohydrates, and 30% as fat (10% SFA, 10% MUFA, 10% PUFA)		Dairy products, meat, and fish	ns
Mofidi et al (2017)[46]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week
Rahimlou et al (2016)[47]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week
Razavi Zade et al (2016)[41]	Hypocaloric	52%–55% of total energy as carbohydrates, 16%–18% as protein, and 30% as fat.	Both diets designed to be calorie-restricted (350–700 kcal deficit) depending on the BMI of the individual. Calorie requirements of each patient estimated based on resting energy expenditure (by use of Harris-Benedict equation) and physical activity levels	Higher intake of whole grains and simple sugar than DASH diet. Moderate fruit, vegetable, and meat, poultry, and fish intake. Low dairy, nuts, and legume intake	Researchers requested participants not to change their routine physical activity and not to consume any supplements and medications that might influence related markers
	DASH	52%–55% of total energy as carbohydrates, 16%–18% as protein, and 30% as fat		Rich in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, cholesterol, refined grains, and sweets. Suggested sodium was <2400 mg/d	
Shahmohammadi et al (2017)[48]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 3 **Continued**

Reference	Diet label	Nutrient composition targets	Caloric intake recommendations	Main food sources	Physical activity recommendations
Sofi et al (2010)[36]	Mediterranean	ns	ns	Dietary recommendations and a package of olive oil not enriched with n-3 PUFA	Participants were asked to indicate their usual pattern of physical activity
	Mediterranean diet + olive oil enriched with n-3 PUFA	ns	ns	Dietary recommendations and a package of olive oil enriched with n-3 PUFA at the dosage of 6.5 mL/d (0.83 g n-3 PUFA, of which 0.47 g eicosapentaenoic acid and 0.24 g docosahexaenoic acid)	
Spadaro et al (2008)[39]	AHA	50% of total energy as carbohydrates, 20% as protein, and 30% as fat	All obese and overweight patients were advised to lose weight with a restriction of daily caloric intake to 25–30 kcal/kg per day	ns	Initially, engaging in a moderate level of physical activity for 30–45 min recommended. Subsequent increases to 30–60 min on most/all days of the week need to be individualized and are targeted to expend a total of 100–200 kcal
Yari et al (2016)[50]	Energy-balanced	<30% of total energy as fat (10% as SFAs, 15% as MUFAs, and 5% as PUFAs), 15%–18% as protein, 52%–55% as carbohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d	≈500 to 1000 kcal/d reduction from usual intake	ns	Patients were also advised to exercise >30 min, 3 times per week

Abbreviations: AHA, American Heart Association; BMI, body mass index; BW, body weight; DASH, Dietary Approaches to Stop Hypertension; HFD, high-fat diet; LF, low fat; MUFA, monounsaturated fatty acid; NCEP, National Cholesterol Education Program; ns, not specified; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 4 **Data extracted for intervention effects of cytokines**

High sensitivity C-reactive protein

Reference	Diet	Pre-intervention, mg/L	Post-intervention, mg/L	*P* value	Change	Mean change (95%CI), mg/mL	Mean change ± SD, mg/L
Dietary intervention alone							
Baldry et al (2017)[38]	Very low energy diet; food based-diet	8.2 (42.8)[a]	5.1 (21.7)[a]	0.007*	−37.8%		
	Very low energy diet; meal-replacement plan	9.6 (29.1)[a]	6.4 (21.8)[a]	0.004*	−33.3%		
Kani et al (2014)[33]	Low calorie	nd	nd	nd			−1.0 ± 0.6
	Low calorie, low carbohydrate	nd	nd	nd			−1.1 ± 0.6
	Low calorie, low carbohydrate, soy containing	nd	nd	0.01*			−8.0 ± 1.0
Marina et al (2014)[51]	LF	3.3 ± 2.8	2.8 ± 2.5	ns	−15.1%		
	HF	2.3 ± 1.9	2.2 ± 1.2	ns	−4.3%		
Razavi Zade et al (2016)[41]	Hypocaloric	4.9 ± 3.4	4.6 ± 2.8	0.08	−6.1%		
	DASH	4.8 ± 3.3	3.6 ± 2.7	0.004*	−25.0%		
Dietary intervention plus co-intervention							
Chan et al (2010)[40]	Hypocaloric, LF	2.2 ± 1.3	2.4 ± 1.6	nd	+9.1%		
	Hypocaloric, LF + cholesterol-lowering agent	3.9 ± 3.8	2.2 ± 2.7	<0.05*	−43.6%		
Kaliora et al (2016)[49]	Isocaloric	2.4 ± 3.0	0.84 ± 1.1	0.023*	−65.0%		
	Isocaloric + Corinthian currants	2.1 ± 1.8	0.82 ± 0.7	0.002*	−60.9%		
Malaguarnera et al (2010)[45]	NCEP	8.7 ± 3.4	7.4 ± 3.2	ns	−14.9%		
	NCEP + L-carnitine	9.1 ± 3.2	5.2 ± 3.1	<0.001*	−42.9%		
Dietary intervention plus supplementation							
Eslamparast et al (2014)[44]	Energy-balanced	nd	nd			−1.04 (−1.5 to −0.6)	
	Energy-balanced + synbiotic supplement	nd	nd			−2.30 (−3.0 to −1.5)	
Mofidi et al (2017)[46]	Energy-balanced	nd	nd				−0.42 ± 0.1[c]
	Energy-balanced + synbiotic supplement	nd	nd				−1.16 ± 0.4[c]
Rahimlou et al (2016)[47]	Energy-balanced	4.8 ± 0.2	2.8 ± 0.2	0.005*	−41.7%		
	Energy-balanced + ginger supplement	4.6 ± 0.1	3.4 ± 0.1	0.007*	−26.1%		
Shahmoham-madi et al (2017)[48]	Energy-balanced	1.5 (0.4, 2.7)[b]	1.5 (0.4, 3.0)[b]	0.846	0.0%		
	Energy-balanced + GCBE supplement	1.4 (0.4, 3.4)[b]	1.1 (0.5, 2.3)[b]	<0.001*	−21.4%		
Yari et al (2016)[50]	Energy-balanced	nd	nd			−1.02 (−1.6 to −0.5)	
	Energy-balanced + Flaxseed supplement	nd	nd			−2.05 (−2.6 to 1.5)	

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

Table 4 **Continued**

Tumor necrosis factor alpha

Reference	Diet	Pre-intervention, ng/mL	Post-intervention, ng/mL	*P* value	Change	Mean change (95%CI), ng/mL
Dietary intervention alone						
Markova et al (2016)[35]	Plant protein isocaloric	4.5 ± 2.6	3.8 ± 2.4	0.016*	−15.6%	
	Animal protein isocaloric	4.3 ± 2.8	4.4 ± 2.2	0.925	+2.3%	
Dietary intervention plus co-intervention						
Chan et al (2010)[46]	Hypocaloric, LF	5.4 ± 1.6	5.4 ± 1.9	ns	0.0%	
	Hypocaloric, LF + cholesterol-lowering agent	6.3 ± 1.9	5.4 ± 2.3	<0.05*	−14.3%	
Kaliora et al (2016)[49]	Isocaloric	1.3 ± 1.0	0.8 ± 0.5	0.004*	−38.5%	
	Isocaloric diet + Corinthian currants	0.9 ± 1.0	1.3 ± 1.4	0.063	+44.4%	
Malaguarnera et al (2010)[45]	NCEP	1.4 ± 0.2	1.3 ± 0.2	ns	−7.1%	
	NCEP + L-carnitine	1.4 ± 0.3	1.1 ± 0.1	<0.001*	−21.4%	
Dietary intervention plus supplementation						
Amanat et al (2017)[42]	Weight management	1.8 ± 2.6	1.8 ± 2.6	0.99	0.0%	
	Weight management + soy isoflavone	1.8 ± 2.5	1.6 ± 2.4	0.01*	−11.1%	
Eslamparast et al (2014)[44]	Energy balanced	nd	nd			−0.59 (−0.8 to −0.3)
	Energy balanced + synbiotic supplement	nd	nd			−1.40 (−1.7 to −1.1)
Yari et al (2016)[50]	Energy-balanced					−0.14 (−0.07 to −0.2)
	Energy-balanced + flaxseed supplement					−1.30 (−0.4 to 2.2)
Mofidi et al (2017)[46]	Energy balanced					−0.30 ± 0.2[a]
	Energy balanced + synbiotic supplement					−1.22 ± 0.8[a]
Rahimlou et al (2016)[47]	Energy-balanced	3.0 ± 0.2	2.8 ± 0.2	0.003	−6.7%	
	Energy-balanced + ginger supplement	4.7 ± 0.4	3.5 ± 0.4	0.00	−25.5%	
Shahmoham-madi et al (2017)[48]	Energy-balanced	8.2 ± 3.2	8.8 ± 4.1	0.279	+7.3%	
	Energy-balanced + GCBE supplement	9.6 ± 3.9	8.6 ± 5.0	0.161	−10.4%	
Spadaro et al (2008)[39]	AHA	3.1 ± 0.4	3.0 ± 0.7	ns	−3.2%	
	AHA + n-3 PUFA supplement	3.3 ± 0.5	2.7 ± 0.5	<0.05	−18.2%	

Interleukin 6

Reference	Diet	Pre-intervention, pg/mL	Post-intervention, pg/mL	*P* value	Change
Dietary intervention alone					
Baldry et al (2017)[38]	Very-low-energy diet; food-based diet	3.7 (10.4)[a]	3.7 (25.4)[a]	0.175	0.0%
	Very-low-energy diet; meal- replacement plan	4.5 (42.6)[a]	3.7 (25.4)[a]	0.040*	−17.8%

(continued)

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Table 4 **Continued**

Interleukin 6

Reference	Diet	Pre-intervention, pg/mL	Post-intervention, pg/mL	*P* value	Change
Marina et al (2014)[51]	LF	1.08 (1.09)[a]	1.01 (1.14)[a]	ns	−6.5%
	HF	0.91 (1.4)[a]	0.83 (2.4)[a]	ns	−8.8%
Markova et al (2016)[35]	Plant protein isocaloric	1.4 ± 1.4	1.4 ± 1.5	0.816	−1.4%
	Animal protein isocaloric	1.1 ± 1.1	0.9 ± 0.7	0.166	−21.7%
Dietary intervention plus co-intervention					
Chan et al (2010)[40]	Hypocaloric, LF	0.8 ± 0.2	0.9 ± 0.4	ns	+12.5%
	Hypocaloric, LF + cholesterol- lowering agent	1.1 ± 0.4	0.9 ± 0.5	<0.05*	−18.2%
Kaliora et al (2016)[49]	Isocaloric	1.7 ± 3.2	1.3 ± 1.4	0.322	−23.5%
	Isocaloric diet + Corinthian currants	1.6 ± 1.4	0.9 ± 0.5	0.009*	−43.7%
Dietary intervention plus supplementation					
Amanat et al (2017)[42]	Weight management	18.2 ± 3.4	18.1 ± 1.8	0.80	0.5%
	Weight management + soy isoflavone	18.8 ± 3.1	16.6 ± 2.5	0.01*	−11.7%

Data presented as mean ± SD or % change (calculated from mean values).

Abbreviations: AHA, American Heart Association; DASH, Dietary Approaches to Stop Hypertension; GCBE, green coffee bean extract; HF, high fat; LF, low fat; nd, no data; ns, not significant; NCEP, National Cholesterol Education Program; PUFA, polyunsaturated fatty acid.

[a]Median (range);

[b]Mean (minimum, maximum);

[c]Mean change ± SEM.

*Statistically significant. $P < 0.05$ significant.

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

Table 5 **Data extracted for intervention effects of adipokines**

Adiponectin					
Reference	Diet	Pre-intervention, ug/mL	Post-intervention, ug/mL	*P* value	Change
Dietary intervention alone					
Marina et al (2014)[51]	LF	3.4 ± 0.94	4.1 ± 3.8	ns	+20.6%
	HF	4.2 ± 2.8	4.6 ± 3.8	ns	+9.5%
Markova et al (2016)[35]	Plant protein isocaloric	4.2 ± 1.7	3.6 ± 1.3	0.003	−14.3%
	Animal protein isocaloric	4.1 ± 3.5	3.6 ± 3.0	ns	−12.2%
Sofi et al (2010)[36]	Mediterranean	1.17 ± 0.08	1.25 ± 0.06	nd	+6.8%
	Mediterranean plus olive oil enriched with n-3 PUFA	1.14 ± 0.02	1.48 ± 0.09	0.04*	+29.8%
Dietary intervention plus co-intervention					
Chan et al (2010)[40]	Hypocaloric, LF	5.9 ± 2.2	6.8 ± 2.5	<0.05*	+15.2%
	Hypocaloric, LF + cholesterol-lowering agent	4.9 ± 2.7	6.1 ± 3.5	<0.05*	+24%
Garinis et al (2010)[32]	Hypocaloric	7.9 ± 4.4	8.5 ± 4.6	0.17	+7.6%
	Hypocaloric + oral hypoglycemic agent	5.8 ± 2.7	7.0 ± 3.3	0.005*	+20.7%
Dietary intervention plus supplementation					
Behrouz et al (2017)[43]	Energy-balanced	25.8 ± 9.4	39.4 ± 24.2	0.005*	+52.7%
	Energy-balanced + probiotic supplement	24.4 ± 11.1	40.7 ± 24.1	<0.001*	+66.8%
	Energy-balanced + prebiotic supplement	27.8 ± 10.4	43.9 ± 15.6	<0.001*	+57.9%

Leptin					
Reference	Diet	Pre-intervention, ng/mL	Post-intervention, ng/mL	*P* value	Change
Dietary intervention alone					
Marina et al (2014)[51]	LF	13.9 ± 10.4	15.1 ± 10.4	ns	+8.6%
	HF	17.3 ± 11.1	16.8 ± 12.6	ns	−2.9%
Dietary intervention plus co-intervention					
Kaliora et al (2016)[49]	Isocaloric	63.5 ± 48.6	55.2 ± 39.4	0.09	−13.1%
	Isocaloric diet + Corinthian currants	95.9 ± 81.6	85.2 ± 76.8	0.19	−11.16%
Dietary intervention plus supplementation					
Behrouz et al (2017)[43]	Energy-balanced	75.8 ± 26.9	74.4 ± 26.2	0.629	−1.8%
	Energy-balanced + probiotic supplement	73.1 ± 26.8	48.6 ± 13.6	<0.001*	−33.5%
	Energy-balanced + prebiotic supplement	80.3 ± 29.7	56.8 ± 22.8	<0.001*	−29.3%

Data presented as mean ± SD or % change (calculated from mean values).
*Statistically significant. $P < 0.05$ significant.
Abbreviations: HF, high fat; LF, low fat; nd, no data; ns, not significant; PUFA, polyunsaturated fatty acid.

hypocaloric, low-fat diet plus a cholesterol-lowering supplement ($P < 0.05$) in comparison with a hypocaloric, low-fat diet alone, which resulted in a nonsignificant increase in hs-CRP (NS). Similarly, an NCEP diet plus L-carnitine supplement significantly reduced hs-CRP ($P < 0.05$) in comparison with the nonsignificant reduction seen in the NCEP diet alone (NS).[45] Significant reductions in hs-CRP occurred after both an energy-balanced diet alone and an energy-balanced diet alongside ginger supplementation (P=0.005 and 0.007, respectively).[47] In contrast, Shahmohammadi et al[48] found an energy-balanced diet alone did not change levels of hs-CRP, whereas an energy-balanced diet plus GCBE supplement improved hs-CRP (P=0.846 and <0.001, respectively). Two studies compared an energy-balanced diet alone with an energy-balanced diet plus synbiotic supplement, and a third study compared an energy-balanced diet with or without flaxseed supplementation; all studies reported a decrease in hs-CRP for all groups, although the mean decrease in supplementation groups were significantly greater ($P < 0.001$).[44,46,50]

Tumor necrosis factor alpha. Ten of the 11 studies that analyzed TNF-α reported significant improvements with dietary interventions, and 1 study reported beneficial change in the supplementation group only, albeit

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

	RELEVANCE				VALIDITY										
Study (Ref)	1	2	3	4	1	2	3	4	5	6	7	8	9	10	RANK
Amanat et al (2017)[42]	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	+
Baldry et al (2017)[38]	Y	Y	Y	Y	Y	Y	Y	Y	N	Ø	Y	Y	Y	Y	+
Behrouz et al (2017)[45]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Chan et al (2010)[40]	Y	Y	Y	Y	Y	Y	Y	Y	Ø	N	Y	Y	Y	Y	+
Eslamparast et al (2014)[44]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Garinis et al (2010)[32]	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Ø	+
Kaliora et al (2016)[49]	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	+
Kani et al (2014)[33]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Kugelmas et al (2003)[34]	Y	Y	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	+
Malaguaranera et al (2010)[48]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Marina et al (2014)[51]	Y	Y	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	+
Markova et al (2016)[35]	Y	Y	Y	Y	Y	Y	Y	Ø	N	Y	Y	Y	Y	Y	+
Mofidi et al (2017)[46]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Rahimlou et al (2016)[47]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Razavi Zade et al (2016)[43]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	+
Shahmohammadi et al (2017)[48]	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	+
Sofi et al (2010)[36]	Y	Y	Y	Y	Y	Y	Y	Ø	Ø	Y	Y	Y	Y	Y	+
Spadaro et al (2008)[39]	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Ø	+
Yari et al (2016)[50]	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	+

Abbreviations: Y; Yes, N; No, Ø; Unclear

Figure 2 **Individual quality assessment of studies according to American Dietetic Association quality checklist.**

without statistical significance (Table 4).[33,35,38–42,44–51] Of the diet-alone studies, Kaliora et al[49] found that TNF-α significantly decreased following an isocaloric diet alone (P=0.004) but adversely increased following an isocaloric diet with the addition of Corinthian currents (P=0.063). Markova et al[35] found significant reductions in TNF-α following a plant protein isocaloric diet and no increase following an animal protein isocaloric diet (P=0.016 and 0.925, respectively). Of the studies implementing a diet alongside supplementation, Chan et al[40] reported significant improvement in TNF-α for the hypocaloric, low-fat diet plus cholesterol-lowering supplement ($P < 0.05$) in comparison with the hypocaloric, low-fat diet alone (NS). Similarly, the NCEP diet plus L-carnitine supplementation significantly reduced TNF-α ($P < 0.001$) compared with the NCEP diet alone (NS).[45] Likewise, Amanat et al[42] found significant reductions of TNF-α following a weight-management diet plus soy isoflavone supplement and no change following a weight-management diet alone (P=0.01 and 0.99, respectively). One study investigating an energy-balanced diet alone compared with an energy-balanced diet plus synbiotic supplementation[44] and 1 study investigating an energy-balanced diet alone compared with an energy-balanced diet plus flaxseed supplement[50] reported a decrease in TNF-α for all groups, although the mean decrease in supplementation groups were significantly greater ($P < 0.001$). Furthermore, both an energy-balanced diet alone and an energy-balanced diet alongside ginger supplementation significantly reduced levels of TNF-α (P=0.003 and <0.001, respectively).[47]

Interleukin 6. Six studies reported on the effects of a dietary intervention on levels of IL-6, with 4 studies reporting significant improvements and 2 studies reporting nonsignificant improvements (Table 4).[33,35,38–42,44–51] Of the diet studies, a 24-week study conducted by Kaliora et al[49] found significant reductions in IL-6 with the isocaloric diet plus Corinthian currants compared with a nonsignificant reduction with isocaloric diet alone (P=0.009 and 0.322, respectively). Of the diet and supplementation studies investigating IL-6, Amanat et al[42] reported significant

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

reductions in IL-6 following a weight-management diet plus soy isoflavone supplement compared with a weight-management diet alone, for which no change was seen (P=0.01 and 0.80, respectively). Chan et al[40] reported significant changes for IL-6 in the hypocaloric, low-fat diet plus cholesterol-lowering supplement group ($P < 0.05$) in comparison with the hypocaloric, low-fat diet alone (NS). Kugelmas et al[34] compared an AHA diet with an AHA diet plus vitamin E supplementation and merged these groups for data analysis (due to small and similar intervention groups), reporting a significant decrease in IL-6 concentration (data not presented in table because numerical values were not provided).

Other cytokines. Interleukins 4, 8, 10, 12, and 18,[35,51] monocyte chemoattractant protein 1 (MCP-1),[35] interferon gamma (IFN-γ),[51] visfatin,[49] and retinol binding protein 4 (RBP-4)[40] were each included in 1 study (see Table S1 in the Supporting Information online). Nuclear factor κB (NF-κB)[44,46] and fetuin A[38,40] were included in 2 studies. An animal protein isocaloric diet resulted in significantly decreased IL-18 ($P < 0.05$).[35] Nuclear factor κB decreased following an energy-balanced diet with and without synbiotic and flaxseed supplementation,[44,46] although the mean decrease in supplementation groups before versus after intervention were significantly greater than for diet alone ($P < 0.001$). A hypocaloric, low-fat diet alone and a hypocaloric, low-fat diet plus cholesterol-lowering supplement significantly lowered both RBP-4 ($P < 0.05$) and fetuin A ($P < 0.05$).[40] Very-low-energy diets in the form of a food-based diet and meal-replacement plan both reduced fetuin A significantly ($P < 0.05$).[38] No significant changes were reported for all other markers ($P > 0.05$).

Adipokines.

The effects of a dietary intervention on adiponectin were investigated in 6 studies,[32,35,36,40,43,51] and 3 studies included leptin.[43,49,51] Data extracted for intervention effects of adipokines within each study are presented in Table 5.[32,35,36,40,43,49,51]

Adiponectin. Of the 6 studies evaluating adiponectin, 5 reported a significant ($P < 0.05$) increase in serum adiponectin levels, suggesting improvement in inflammatory status, and 1 study showed no significant ($P > 0.05$) change (Table 5).[32,35,36,40,43,49,51] Of the diet-alone studies, Markova et al[35] reported a significant improvement in adiponectin following a plant protein isocaloric diet (P=0.003) but not an animal protein isocaloric diet (NS). Moreover, Sofi et al[36] observed a significant increase of adiponectin levels in the Mediterranean diet enriched with n-3 PUFA olive oil (P=0.04), whereas a nonsignificant increase was reported for the Mediterranean diet alone (NS). Of the dietary intervention plus supplementation studies, Behrouz et al[43] reported a significant increase in adiponectin for each of the energy-balanced diets alone, the energy-balanced diet plus probiotic, and the energy-balanced diet plus prebiotic groups (P=0.005, <0.001, and 0.001, respectively). A study of a hypocaloric, low-fat diet plus placebo and a hypocaloric, low-fat diet plus cholesterol-lowering agent found that adiponectin increased significantly in both groups ($P < 0.05$).[40] Garinis et al[40] showed that a hypocaloric diet alone compared with a hypocaloric diet plus oral hypoglycemic supplement increased adiponectin for both groups, although the increase reached statistical significance in the hypocaloric diet plus oral hypoglycemic agent group ($P < 0.005$) and not in the hypocaloric diet-only group ($P < 0.17$).

Leptin. Behrouz et al[43] reported significant reductions in leptin following both an energy-balanced diet plus probiotic supplement ($P < 0.001$) and an energy-balanced diet plus prebiotic supplement ($P < 0.001$), although no significant changes were seen following the diet-alone group ($P > 0.05$).

Liver imaging and histology

Five studies assessed liver imaging and histology after intervention using abdominal ultrasound,[32,36,39,41,48] 1 study used ultrasound and transient elastography (TE) Fibroscan,[49] 4 studies used TE Fibroscan only,[44,46,47,50] 3 used ^{1}H-MRS,[35,40,51] and 2 performed liver biopsy.[38,45] Of the 19 studies, 4 did not assess postintervention liver imaging or histology. Data extracted for each of these measures are presented in Table S2 (see Table S2 in the Supporting Information online). Most significant changes occurred following a hypocaloric diet with and without an oral hypoglycemic agent ($P < 0.029$ and $P < 0.0001$),[32] hypocaloric diet with and without a cholesterol-lowering agent ($P < 0.05$),[40] hypocaloric and DASH diet(s) alone ($P < 0.001$),[41] isocaloric diet with and without current supplementation ($P < 0.05$),[49] or energy-balanced dietary intervention alone or with synbiotic,[44,46] ginger,[47] or flaxseed[50] supplementation. The Mediterranean[36] and AHA[39] diets (with or without n-3 PUFA supplement) have also achieved significant reductions in hepatic steatosis and insulin resistance in an NAFLD population, although P values were not reported. Using liver biopsy, the NCEP diet alone significantly reduced NASH activity scores ($P < 0.001$), as did the NCEP diet plus L-carnitine supplementation ($P < 0.001$).[45]

Downloaded from https://academic.oup.com/nutrit/nrreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540795 by La Trobe University Wodonga Campus Library user on 01 August 2019

Quality assessment of studies

The quality assessment of studies using the American Dietetic Association Quality Criteria Checklist for Primary Research[31] is presented in Table 2[32–36,38–45,47–51], and the assessment of internal and external biases of each study is shown in Figure 2.[32–36,38–51] All studies were, overall, found to be of positive (+) quality, with 7 of the 20 studies ranking positive in all sections.[33,41,43–47] Ten studies ranked negative (−) or unclear (ø) due to inadequate blinding of participants or research personnel.[32,34–36,38–40,49–51] Blinding is often not possible in dietary intervention trials; however, blinding of outcome assessors, technicians, and laboratory staff enhances research rigor if applied to all trials. This intent was not clear in the above studies that ranked negative for this domain. Of the aforementioned 11 studies, 6 ranked negative (−) or unclear (ø) in the way they described withdrawals,[34–36,42,48,51] and a further 2 had groups that were considered noncomparable and may affect interpretation of outcome measures due to significant ($P < 0.05$) differences at baseline.[34,51]

Of the 19 included studies, only 7 studies[33,36,38,39,41–43] calculated sample size using statistical power generated to see a significant change, although these outcomes were not specific to inflammatory markers. Furthermore, it was unclear in most studies whether the inflammatory marker(s) were examined as a primary or secondary outcome.

DISCUSSION

This systematic review provides evidence that dietary interventions implemented in RCTs can lower levels of circulating serum inflammatory cytokines and increase levels of circulating adiponectin in individuals with NAFLD. Although the effects of dietary interventions on inflammatory markers varied, diets that demonstrated more favorable change were those that were calorie restricted, those that were isocaloric, and those that adhere to DASH or NCEP dietary guidelines. Dietary interventions with the addition of a co-intervention, specifically nutraceuticals or a pharmacological supplementation, demonstrated added benefits compared with diet alone in an NAFLD population.

In this review, the most effective studies were calorie-restricted dietary interventions that resulted in significant ($P < 0.05$) weight loss. Typically in the treatment of NAFLD weight loss is considered a primary focus because restriction of energy intake induces rapid adipose tissue reduction, thus lowering IR and hepatic steatosis.[53,54] Adipokine and cytokine production is inhibited subsequent to the decrease in adiposity.[55] Although clinical trials investigating calorie-restricted diets report inflammatory changes following weight loss, due to their restrictive nature these diets are often unsustainable in NAFLD patients and may result in portal fibrosis or necroinflammation following rapid weight loss.[56]

This review also highlighted the effects of the NCEP diet, which is advocated in NAFLD to balance macronutrient intake and anti-inflammatory foods, and the DASH eating plan, recommended as a low-glycemic-index, low energy-dense diet with an emphasis on reduced sodium intake. In trials, the NCEP diet has been successful in lowering CRP,[53] as well as hepatic steatosis and fibrosis.[45] The DASH diet has also reduced CRP levels in adults with NAFLD,[41] adolescents with metabolic syndrome,[57] and patients with polycystic ovary syndrome[58]—chronic diseases in which insulin resistance, obesity, and abdominal fat accumulation are underlying pathophysiological contributors. These changes have been attributed to weight loss, considering a reduction in adipocytes accompanied by a reduction in IL-6 is likely to be responsible for the reduction in CRP.[59]

One small study included in this review investigated the Mediterranean diet; researchers did not find a substantial effect for diet alone in an NAFLD population.[36] In this study, however, an improvement in adiponectin was seen following a Mediterranean diet with n-3 enriched olive oil supplementation.[36] Adhering to a diet rich in antioxidants and phenolic compounds from whole grains, fruits, vegetables, nuts, and extra-virgin olive oil may decrease hs-CRP, as well as circulating levels of free radicals and pro-inflammatory cytokines IL-6, IL-18, and TNF-α.[19] These dietary components, typical of the Mediterranean diet, are extensively investigated in the treatment of IR and metabolic syndrome.[19,23] Moreover, Kaliora et al[49] found that within a Greek population, adherence to a Mediterranean diet supplemented with Corinthian currents as a regular dietary snack was associated with an improvement in levels of hs-CRP and IL-6. This was not unprecedented as the authors noted recent studies identifying bioactive phytochemicals and phenolic compounds in currents could potentially ameliorate fasting glucose, inflammation, and the fibrosis stage.[49]

A diet receiving considerable attention in recent RCTs for NAFLD populations and within this review was an energy-balanced diet; implementing "general tips for healthy eating," low-fat cooking methods, and moderate PA recommendations,[52] this diet improved hs-CRP, TNF- α, and adiponectin. Improvements in these inflammatory markers were further enhanced when an energy-balanced diet was combined with prebiotic,[43] probiotic,[43] synbiotic,[44,46] ginger,[47] flaxseed,[50]

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

or GBCE[48] supplements. Although the efficacy of dietary intervention was partially assessed in these studies, the effect of supplementation was considered the primary outcome and found to elicit superior benefits than diet alone. Hence the diet-alone group was used as a control rather than as an experimental group, although noteworthy effects were seen following diet only. Shahmohammadi et al[48] attributed a significant ($P < 0.05$) decrease in hs-CRP to the anti-inflammatory and antioxidant activities of a GCBE supplement. Similarly, Rahimlou et al[47] found their results to be in line with previous studies reporting that ginger supplementation exhibited antidiabetic, anticancer, and anti-inflammatory properties, leading to a significant ($P < 0.05$) decrease in serum levels of TNF- α and hs-CRP.[60,61] Flaxseed oil, a supplement that has been shown to have potential health benefits for cardiovascular disease, metabolic syndrome, and dyslipidemia,[62–64] is thought to improve weight management, lipid profile, IR, and the inflammatory cytokines hs-CRP and TNF-α.[50] Given that flaxseed is a rich source of n-3 fatty acids, it's mechanism of action is to ameliorate hepatic lipid accumulation and oxidative stress. This review found improvements in both leptin and adiponectin following prebiotic, probiotic, and synbiotic supplementation.[43] Few studies have investigated the effects of prebiotics and probiotics in adipokines in humans, although evidence is mounting for potential use of synbiotic supplements to protect the liver from damage. It is thought that synbiotic supplements retard inflammation, resulting in downregulation of insulin signaling in adipose tissue, thereby decreasing fat accumulation. Animal models have displayed the benefits of probiotics on leptin.[65] Moreover, a recent meta-analysis found that microbial therapies of prebiotic, probiotic, and synbiotic supplementation did not improve levels of CRP and TNF-α.[66] L-Carnitine supplementation was seen to have beneficial effects on the inflammatory cytokines hs-CRP and TNF-α[45]; although this has been confirmed in an animal model,[27] human studies in an NAFLD population have yet to prove L-carnitine as a convincing therapeutic option.[25]

Alternatively, Kuglemas et al[34] concluded that lifestyle modification and exercise were associated with improvement in liver enzymes and cholesterol in patients with NASH, whereas vitamin E supplementation provided no apparent added benefit. Previous studies in NAFLD have shown the potential beneficial effects of vitamin E[67] and nutraceutical supplementation on hepatic outcomes when administered alongside diet[45]; however, additional evidence is required before prescription can be recommended for the alleviation of inflammatory outcomes. Whether participants in supplement arms of trials adhere better to the intervention is difficult to determine, as is the efficacy of these therapies alongside diet. The effect of nutraceutical intervention in NAFLD has the potential to be further investigated in a short- to medium-term capacity. However, in this review supplements were only included if they were within an intervention that had a stand-alone dietary intervention arm.

Physical activity, although not a primary outcome of this review, also plays a central role in the alleviation of hepatic and inflammatory outcomes and may independently reduce disease severity.[68] The majority of studies in this review recommended that all study participants, regardless of their assigned intervention group, engage in moderate PA for 30 minutes >3 times per week. Recommendations were brief and generally advised low- to moderate-intensity aerobic exercise and routine stretching. Although PA recommendations were given, adherence to this parameter was not recorded or reported; hence these changes could not be assessed. In future studies, PA should be monitored and/or controlled for so that the true impact of dietary intervention can be assessed.

The use of pharmaceuticals is also emerging in NAFLD. Chan et al[40] showed that ezetimibe, a potent cholesterol absorption inhibitor, improved adiponectin, hs-CRP, TNF-α, and IL-6. The underlying mechanism of ezetimibe is to reduce low-density lipoprotein cholesterol concentrations and therefore improve dyslipidemia. For this reason, it was thought to be an optimal approach in the clinical setting, as well as for moderation of weight loss. Additional studies have found improvement in weight loss when ezetimibe was combined with statins.[34,69,70] Definitive conclusions for ezetimibe cannot be drawn yet due to insufficient evidence surrounding the effects for short- and long-term use.

Although it was not a primary outcome of this review, noteworthy changes in liver histology were evident following hypocaloric,[32,41,45] isocaloric,[49] energy-balanced,[44,46,47,50] DASH,[41] AHA,[39] NCEP,[45] and Mediterranean[36] diets. The addition of various co-interventions resulted in prominent changes in markers of steatosis and fibrosis, as defined by abdominal ultrasound, ^{1}H-MRS, TE Fibroscan, and/or liver biopsies. Changes in liver severity were difficult to compare among studies due to the various liver imaging and histology tools, although findings are relatively consistent with previous literature. Although liver biopsy remains the gold-standard approach in confirming NAFLD severity, the approach remains too invasive, particularly in large dietary intervention cohorts of patients with simple steatosis. Therefore, additional large studies of this disease cohort are required to elucidate the specificity of cytokines and adipokines as surrogate markers of

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

disease. Given the pathophysiology and underlying mechanisms of the chronic inflammatory state of NAFLD, it is important to consider the inflammatory markers presented in this review and the role they place in disease progression in the absence of any known liver-sensitive markers.

This review highlights the limited evidence that is currently available to assess the impact of optimal dietary composition on pro-inflammatory cytokines and adipokines in an NAFLD population. A pooled estimate of effect, or meta-analysis, was not possible given the heterogeneity of control and experimental groups within each study. The populations across the studies were diverse, and the impact of habitual diets and genetics may influence the extent of response to dietary interventions. Other limitations of this review include the small sample size of included studies, reducing statistical power for inflammatory markers as a primary outcome, especially when some inflammatory markers may be more susceptible to change with diet and other external factors. Two studies[40,51] included in this review focused on recruiting obese individuals, of whom 10 participants did not have NAFLD intrahepatic triglyceride content < 5%. Some studies did not report a macronutrient breakdown of the recommended diets; therefore it was difficult to make comparisons or pool together dietary prescriptions. Dietary compliance was often not monitored or reported, and there was inconsistency in regards to cytokines and adipokines studied.

Still this systematic review study has important strengths in that the overall population within the included studies—age, sex, anthropometry, and general characteristics—were reflective of and therefore generalizable to the NAFLD population. Moreover, liver biopsy, ultrasound, magnetic resonance spectroscopy, TE, and/or liver chemistries were used in the diagnosis and reporting of NAFLD in all included studies.

To determine whether dietary interventions, with or without co-interventions, are effective at improving inflammatory outcomes in individuals with NAFLD and to more widely assess liver outcomes, future research should involve large, statistically powered cohorts with specific pro-inflammatory cytokines and adipokines as primary outcome measures in patients with biopsy- or ultrasound-proven NAFLD.[16] Dietary interventions should consist of an experimental diet in comparison with a control (or habitual) diet for the same duration of time. To determine whether dietary interventions are effective at improving inflammatory outcomes, supplementation should not be administered in either group because it will allow the dietary interventions with quality of diet or active nutrients of interest to be adequately assessed. It will also be beneficial, from a mechanistic and clinical standpoint, to distinguish between the effect of diet on serum cytokines and adipokines in the absence of weight loss.

CONCLUSION

Dietary interventions including hypocaloric diets, isocaloric diets, or diets that adhere to DASH or NCEP dietary guidelines appear to demonstrate improvements in circulating serum inflammatory cytokines and adipokines in an NAFLD population. However, these effects were predominantly driven by weight loss. Dietary interventions, including nutraceutical or pharmacological supplementation, appear to elicit superior outcomes compared with diet alone in patients with NAFLD.

Acknowledgments

The authors would like to acknowledge Dr George Moschonis for critically reviewing the manuscript and the Librarian Team at Alfred Health, VIC for their assistance.

Author contributions. A.J.R. and A.C.T. conceptualized and designed this review. A.J.R. and E.S.G. conducted the search process and data extraction. A.J.R., E.S.G., and A.C.T. contributed to data analysis and interpretation. A.J.R. drafted the manuscript, and all authors reviewed and approved the final manuscript.

Funding. This work was supported by an Australian Government Research Training Program Scholarship (to A.J.R.). No external funding was received for this work. No authors are affiliated with or have received funding from companies responsible for the pharmacological or nutraceutical agents, devices, or medical technology identified and discussed in this manuscript.

Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA checklist**

Appendix S2 **Search strategy**

Table S1 **Data extracted for intervention effects of other cytokines**

Table S2 **Data extracted for intervention effects on liver histology and imaging**

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

REFERENCES

1. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol*. 2013;10:686–690.
2. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of non-alcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence and outcomes. *Hepatology*. 2015;64:73–84.
3. Sofi F, Casini A. Mediterranean diet and non-alcoholic fatty liver disease: new therapeutic option around the corner. *World J Gastroenterol*. 2014;20:7339–7346.
4. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology*. 2015;148:547–555.
5. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;52:1836–1846.
6. Gallic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol*. 2010;316:129–139.
7. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. 2004;92:347–355.
8. Lade A, Noon LA, Friedman SL. Contributions of metabolic dysregulation and inflammation to nonalcoholic steatohepatitis, hepatic fibrosis, and cancer. *Curr Opin Oncol*. 2014;26:100.
9. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care*. 2007;30:1212–1218.
10. Barrera F, George J. The role of diet and nutritional intervention for the management of patients with NAFLD. *Clin Liver Dis*. 2014;18:91–112.
11. George ES, Forsyth A, Itsiopoulos C, et al. Practical dietary recommendations for the prevention and management of nonalcoholic fatty liver disease in adults. *Adv Nutr*. 2018;9:30–40.
12. George ES, Tierney AC, Campbell KL, et al. What is the optimal dietary composition for NAFLD? *Curr Hepatol Rep*. 2017;16:346–355.
13. Auwerx J, Staels B. Leptin. *Lancet*. 1998;351:737–742.
14. Chitturi S, Farrell G, Frost L, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology*. 2002;36:403–409.
15. Harrison SA, Day CP. Benefits of lifestyle modification in NAFLD. *Gut*. 2007;56:1760–1769.
16. Papamiltiadous ES, Roberts SK, Nicoll AJ, et al. A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA): study protocol. *BMC Gastroenterol*. 2016;16:14.
17. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Obes Facts*. 2016;9:65–90.
18. Dowman JK, Tomlinson J, Newsome P. Pathogenesis of non-alcoholic fatty liver disease. *QJM*. 2010;103:71–83.
19. Stedman N, Hohmann C-D, Kessler C, et al. Effects of different dietary approaches on inflammatory markers in patients with metabolic syndrome: a systematic review and meta-analysis. *Nutrition*. 2016;32:338–348.
20. Abenavoli L, Milic N, Peta V, et al. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. *WJG*. 2014;20:16831–16840.
21. Musso G, Gambino R, Cassader M, et al. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology*. 2010;52:79–104.
22. Nordmann AJ, Suter-Zimmermann K, Bucher HC, et al. Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. *Am J Med*. 2011;124:841–851.e842.
23. Suárez M, Boqué N, del Bas JM, et al. Mediterranean diet and multi-ingredient-based interventions for the management of non-alcoholic fatty liver disease. *Nutrients*. 2017;9:1052.
24. Yki-Järvinen H. Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. *Nutrients*. 2015;7:9127–9138.
25. Del Ben M, Polimeni L, Baratta F, et al. The role of nutraceuticals for the treatment of non-alcoholic fatty liver disease. *Br J Clin Pharmacol*. 2017;83:88–95.
26. Shatifi N, Amani R, Hajiani E, et al. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocrine*. 2014;47:70–80.
27. Somi MH, Fatahi E, Panahi J, et al. Data from a randomized and controlled trial of LCarnitine prescription for the treatment for non-alcoholic fatty liver disease. *BioInformation*. 2014;10:575.
28. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med*. 2009;151:W-65–W-94.
29. Higgins JP, Green S. *Cochrane Handbook for Systematic Reviews of Interventions*. Vol 5. London: Wiley Online Library; 2008.
30. Stone PW. Popping the (PICO) question in research and evidence-based practice. *Appl Nurs Res*. 2002;15:197–198.
31. Association AD. Evidence Analysis Manual: Steps in the Academy Evidence Analysis Process. Chicago: American Dietetic Association; 2012.
32. Gastinis GA, Fruci B, Mazza A, et al. Metformin versus dietary treatment in nonalcoholic hepatic steatosis: a randomized study. *Int J Obes*. 2010;34:1255–1264.
33. Kani AH, Alavian SM, Esmaillzadeh A, et al. Effects of a novel therapeutic diet on liver enzymes and coagulating factors in patients with non-alcoholic fatty liver disease: a parallel randomized trial. *Nutrition*. 2014;30:814–821.
34. Kugelmas M, Hill DB, Vivian B, et al. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology*. 2003;38:413–419.
35. Markova M, Pivovarova O, Hornemann S, et al. Isocaloric diets high in animal or plant protein reduce liver fat and inflammation in individuals with type 2 diabetes. *Gastroenterology*. 2016;152:571–585.
36. Sofi F, Giangrandi I, Cesari F, et al. Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. *Int J Food Sci Nutr*. 2010;61:792–802.
37. Ekhlasi G, Shidfar F, Agah S, et al. Effects of pomegranate and orange juice on antioxidant status in non-alcoholic fatty liver disease patients: a randomized clinical trial. *Int J Vitam Nutr Res*. 2016;85:292–298.
38. Baldry EL, Aithal GP, Kaye P, et al. Effects of short-term energy restriction on liver lipid content and inflammatory status in severely obese adults: results of a randomized controlled trial using 2 dietary approaches. *Diabetes Obes Metab*. 2017;19:1179–1183.
39. Spadaro L, Magliocco O, Spampinato D, et al. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. *Dig Liver Dis*. 2008;40:194–199.
40. Chan DC, Watts GF, Gan SK, et al. Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care*. 2010;33:1134–1139.
41. Razavi Zade M, Telkabadi MH, Bahmani F, et al. The effects of DASH diet on weight loss and metabolic status in adults with non-alcoholic fatty liver disease: a randomized clinical trial. *Liver Int*. 2016;36:563–571.
42. Amanat S, Eftekhari MH, Fararouei M, et al. Genistein supplementation improves insulin resistance and inflammatory state in non-alcoholic fatty liver patients: a randomized, controlled trial. *Clin Nutr*. 2017;37:1210–1215.
43. Behrouz V, Jazayeri S, Aryaeian N, et al. Effects of probiotic and prebiotic supplementation on leptin, adiponectin, and glycemic parameters in non-alcoholic fatty liver disease: a randomized clinical trial. *Middle East J Dig Dis*. 2017;9:150.
44. Eslamparast T, Poustchi H, Zamani F, et al. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr*. 2014;99:535–542.
45. Malaguarnera M, Gargante MP, Russo C, et al. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis–a randomized and controlled clinical trial. *Am J Gastroenterol*. 2010;105:1338–1345.
46. Mofidi F, Poustchi H, Yari Z, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. *Br J Nutr*. 2017;117:662–668.
47. Rahimlou M, Yari Z, Hekmatdoost A, et al. Ginger supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Hepat Mon*. 2016;16:e34897.
48. Shahmohammadi HA, Hosseini SA, Hajiani E, et al. Effects of green coffee bean extract supplementation on patients with non-alcoholic fatty liver disease: a randomized clinical trial. *Hepat Mon*. 2017;17:e45609.
49. Kallios AC, Kokkinos A, Diolintzi A, et al. The effect of minimal dietary changes with raisins in NAFLD patients with non-significant fibrosis: a randomized controlled intervention. *Food Funct*. 2016;7:4533–4544.
50. Yari Z, Rahimlou M, Eslamparast T, et al. Flaxseed supplementation in non-alcoholic fatty liver disease: a pilot randomized, open labeled, controlled study. *Int J Food Sci Nutr*. 2016;67:461–469.
51. Marina A, von Frankenberg AD, Suvag S, et al. Effects of dietary fat and saturated fat content on liver fat and markers of oxidative stress in overweight/obese men and women under weight-stable conditions. *Nutrients*. 2014;6:4678–4690.
52. National Institutes of Health. National Heart Lung, and Blood Institute. The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. NIH Publication No. 00-4084. Bethesda, MD: National Institutes of Health, National Heart Lung, and Blood Institute; 2000.
53. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. *Arterioscler Thromb Vasc Biol*. 2006;26:995–1001.
54. Naniwadekar AS. Nutritional recommendations for patients with non-alcoholic fatty liver disease: an evidence based review. *Pract Gastroenterol*. 2010;82:8–16.
55. Jarrar M, Baranova A, Collantes R, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2008;27:412–421.
56. Andersen T, Gluud C, Franzmann M-B, et al. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol*. 1991;12:224–229.
57. Vollmer WM, Sacks FM, Ard J, et al. Effects of diet and sodium intake on blood pressure: subgroup analysis of the DASH-sodium trial. *Ann Intern Med*. 2001;135:1019–1028.

Downloaded from https://academic.oup.com/nutritionreviews/advance-article-abstract/doi/10.1093/nutrit/nuz029/5540705 by La Trobe University Wodonga Campus Library user on 01 August 2019

58. Asemi Z, Esmaillzadeh A. DASH diet, insulin resistance, and serum hs-CRP in polycystic ovary syndrome: a randomized controlled clinical trial. *Horm Metab Res*. 2015;47:232–238.
59. Dietrich M, Jialal I. The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. *Nutr Rev*. 2005;63:22–28.
60. Bhandari U, Pillai K. Effect of ethanolic extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. *J Ethnopharmacol*. 2005;97:227–230.
61. Thomson M, Al-Qattan K, Al-Sawan S, et al. The use of ginger (*Zingiber officinale Rosc.*) as a potential anti-inflammatory and antithrombotic agent. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:475–478.
62. Leyva DR, Zahradka P, Ramjiawan B, et al. The effect of dietary flaxseed on improving symptoms of cardiovascular disease in patients with peripheral artery disease: rationale and design of the FLAX-PAD randomized controlled trial. *Contemp Clin Trials*. 2011;32:724–730.
63. Pan A, Yu D, Demark-Wahnefried W, et al. Meta-analysis of the effects of flaxseed interventions on blood lipids. *Am J Clin Nutr*. 2009;90:288–297.
64. Rhee Y, Brunt A. Flaxseed supplementation improved insulin resistance in obese glucose intolerant people: a randomized crossover design. *Nutr J*. 2011;10:44.
65. Takemura N, Okubo T, Sonoyama K. *Lactobacillus plantarum* strain no. 14 reduces adipocyte size in mice fed high-fat diet. *Exp Biol Med (Maywood)*. 2010;235:849–856.
66. Loman BR, Hernández-Saavedra D, An R, et al. Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Nutr Rev*. 2018;76:822–839.
67. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. 2010;362:1675–1685.
68. Kistler KD, Brunt EM, Clark JM, et al. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol*. 2011;106:460–468.
69. Deushi M, Nomura M, Kawakami A, et al. Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome. *FEBS Lett*. 2007;581:5664–5670.
70. Sager PT, Capece R, Lipka L, et al. Effects of ezetimibe coadministered with simvastatin on C-reactive protein in a large cohort of hypercholesterolemic patients. *Atherosclerosis*. 2005;179:361–367.

Downloaded from https://academic.oup.com/nutrit/nuz029/5540705 by LaTrobe University Wodonga Campus Library user on 01 August 2019

Appendix 2. Food parameters included in the dietary inflammatory index, inflammatory effect scores and intake values from the global composite data set; Dietary Inflammatory Index Development Study, Columbia, SC, USA, 2011-2012[1]

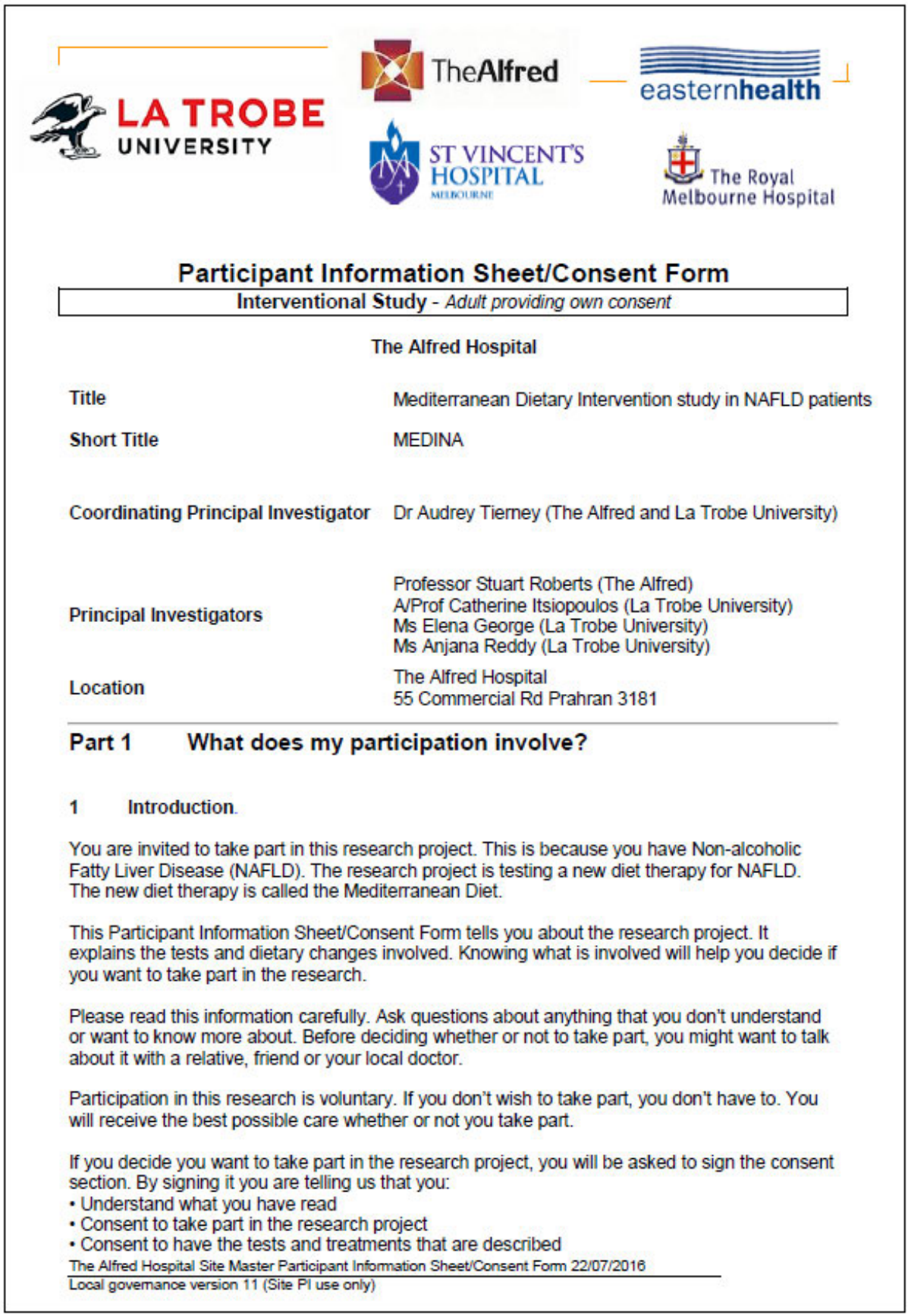
Food Parameter	Weighted number of articles	Raw inflammatory effect score*	Overall inflammatory effect score†	Global daily mean intake (units/day)‡	SD‡
Alcohol (g)	417	-0.278	-0.278	13.98	3.72
Vitamin B12 (µg)	122	0.205	0.106	5.15	2.70
Vitamin B6 (mg)	227	-0.379	-0.365	1.47	0.74
Beta Carotene (µg)	401	-0.584	-0.584	3718	1720
Caffeine (g)	209	-0.124	-0.110	8.05	6.67
Carbohydrate (g)	211	0.180	0.180	272.2	40.0
Cholesterol (mg)	75	0.347	0.110	279.4	51.2
Energy (kcal)	245	0.180	0.180	2056	338
Eugenol (mg)	38	-0.868	-0.140	0.01	0.08
Fat (g)	443	0.298	0.298	71.4	19.4
Fibre (g)	261	-0.663	-0.663	18.8	4.9
Folate (µg)	217	-0.207	-0.190	273.0	70.7
Garlic (g)	277	-0.412	-0.412	4.35	2.90
Ginger (g)	182	-0.588	-0.453	59.0	63.2
Iron (mg)	619	0.032	0.032	13.35	3.71
Magnesium (mg)	351	-0.484	-0.484	310.1	139.4
MUFA (g)	106	-0.019	-0.009	27.0	6.1
Niacin (mg)	58	-1.000	-0.246	25.90	11.77
Omega 3 (g)	2588	-0.436	-0.436	1.06	1.06
Omega 6 (g)	924	-0.159	-0.159	10.8	7.50
Onion (g)	145	-0.490	-0.301	35.9	18.4
Protein (g)	102	0.049	0.021	79.4	13.9
PUFA (g)	4002	-0.337	-0.337	13.88	3.76
Riboflavin (mg)	22	-0.727	-0.068	1.70	0.79
Saffron (g)	33	-1.000	-0.140	0.37	1.78
Saturated fat (g)	205	0.429	0.373	28.6	8.0
Selenium (µg)	372	-0.191	-0.191	67.0	25.1
Thiamin (mg)	65	-0.354	-0.098	1.70	0.66
Trans fat (g)	125	0.432	0.229	3.15	3.75
Turmeric (mg)	814	-0.785	-0.785	533.6	754.3
Vitamin A (RE)	663	-0.401	-0.401	983.9	518.6
Vitamin C (mg)	733	-0.424	-0.424	118.2	43.46
Vitamin D (µg)	996	-0.446	-0.446	6.26	2.21
Vitamin E (mg)	1495	-0.419	-0.419	8.73	1.49
Zinc (mg)	1036	-0.313	-0.313	9.84	2.19
Green/black tea (g)	735	-0.536	-0.536	1.69	1.53
Flavan-3-ol (mg)	521	-0.415	-0.415	95.8	85.9
Flavones (mg)	318	-0.616	-0.616	1.55	0.07
Flavonols (mg)	887	-0.467	-0.467	17.70	6.79
Flavonones (mg)	65	-0.908	-0.250	11.70	3.82
Anthocyanidins (mg)	69	-0.449	-0.131	18.05	21.14
Isoflavones (mg)	484	-0.593	-0.593	1.20	0.20
Pepper (g)	78	-0.397	-0.131	10.00	7.07
Thyme/oregano (mg)	24	-1.000	-0.102	0.33	0.99
Rosemary (mg)	9	-0.333	-0.013	1.00	15.00

[1]Shivappa, et al. (2014). Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr, 17(08), 1689-1696.

SD, standard deviation; RE, retinol equivalents. *Food parameter-specific raw inflammatory effect score. Note that the effect is per unit amount noted for each food parameter. †Food parameter-specific overall inflammatory effect score accounting for the robustness of the literature, which is considered optimal at the median of 236 articles. ‡From the world composite database.

Appendix 3 Participant Information Consent Form (PICF) and Common Research Forms (CRF's)

3.1 PICF



LA TROBE UNIVERSITY

TheAlfred

easternhealth

ST VINCENT'S HOSPITAL MELBOURNE

The Royal Melbourne Hospital

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

The Alfred Hospital

Title	Mediterranean Dietary Intervention study in NAFLD patients
Short Title	MEDINA
Coordinating Principal Investigator	Dr Audrey Tierney (The Alfred and La Trobe University)
Principal Investigators	Professor Stuart Roberts (The Alfred) A/Prof Catherine Itsiopoulos (La Trobe University) Ms Elena George (La Trobe University) Ms Anjana Reddy (La Trobe University)
Location	The Alfred Hospital 55 Commercial Rd Prahran 3181

Part 1 What does my participation involve?

1 Introduction.

You are invited to take part in this research project. This is because you have Non-alcoholic Fatty Liver Disease (NAFLD). The research project is testing a new diet therapy for NAFLD. The new diet therapy is called the Mediterranean Diet.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and dietary changes involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described

• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

The aim of this study is to determine if the Mediterranean Diet (MD) compared to standard care (low fat diet), can reduce the severity of NAFLD and other symptoms often associated with NAFLD namely the Metabolic Syndrome (MetS). The components of the MetS which this study aims to monitor and potentially reduce includes: high blood pressure, increased waist circumference, elevated triglycerides (bad fats in the blood), reduced HDL cholesterol (good fats in the blood) and elevated glucose levels. These symptoms are referred to as the Metabolic Syndrome (MS).

What is the current treatment available for NAFLD
Currently the only way to treat NALFD is through weight reduction and this can be difficult to achieve and even harder to maintain. This study is investigating whether the MD is able to improve NAFLD and MetS without weight loss.
If this research is shown to be successful it will provide a treatment option for the many people who have NAFLD and the MetS.

Why are we testing the Mediterranean Diet in NAFLD patients?
The MD has already been shown to be effective in helping prevent heart disease and in improving diabetes. The reason why the MD has been selected for testing in NAFLD patients is because a small preliminary study conducted in Melbourne reported that the diet was successful in reducing liver fat and improving insulin sensitivity in this group. The current study will help contribute to the evidence which will help to determine if this diet should be recommended by dietitians and clinicians in this patient group as a treatment method.

The Mediterranean diet is an experimental diet. This means that it is not currently the recommended diet for NAFLD in Australia because there is not enough evidence.

Will this research be a part of obtaining an educational qualification?
The results of this research will be used by the study dietitian Elena George and researcher Anjana Reddy to obtain their PhD degrees. This research will also be used to help fulfil the requirements of honours student research projects.

Who is coordinating this research?
This research is being conducted by La Trobe University in collaboration with The Alfred, St Vincent's, Box hill and The Royal Melbourne Hospitals.

3 What does participation in this research involve?

You will be participating in a randomised controlled research project. Sometimes we do not know which diet is best for treating a condition. To find out we need to compare different diets. We put people into groups and give each group a different diet. The results are compared to see if one is better. To try to make sure the groups are the same, each participant is put into a group by chance (random). There are two groups: the control (standard care, a low fat diet) and the intervention group (Mediterranean diet). You have a 50% chance to be randomly placed into either.

Consent
There will be no study assessments performed unless consent has been given in written form first. The study will be explained to you and once you have understood and agree that you would like to take part in the study you will be screened for eligibility.

Screening Process

The screening process will be carried out face to face at the Alfred and will take about 15-20 minutes. The process involves answering a series of questions, if these questions meet the eligibility criteria you will be randomised into either the control or intervention group, then you will be booked in for your first appointment.

All appointments will be carried out at The Alfred hospital except if you choose to be involved in the DEXA Scans. Please see "DEXA Scan Volunteers" towards the end of this section for more information.

First Appointment
Before your first appointment you will be asked to complete a number of documents to bring along. These include: 3 day food diary (2 week days and 1 weekend), Food Frequency Questionnaire (FFQ), Physical Activity Questionnaire and a Short Form (SF-36). At the first appointment you will be told which group you are in and when you will be undertaking MR-S imaging. You will have your first of five face to face consultations at The Alfred. You will be asked to be fasted for the purpose of the blood test). Upon arrival first you will provide a 'first morning' urine sample and then the research team will take a blood test.

You will also undergo Magnetic Resonance Spectroscopy (MR-S) imaging at Baker IDI (an independent institution), this appointment could be on the same day as your other study appointments or on another day within a week of your appointment. Here you will be asked to lie still for approximately 20-30 minutes so that images of your liver can be taken. The procedure requires a voxel to be placed on the abdomen over the liver.

Finally you will attend a consultation with the dietitian. During the consultation there will be a series of measures taken. These include: weight, height, waist circumference, hip circumference, neck circumference, body composition (Bioelectrical Impedance Analysis), and blood pressure. We will also take a once off cheek swab for genetic analysis. This process involves collecting some cells from inside your mouth with a cotton tip; the process is painless and takes about 60 seconds.
You will then be educated on either a low fat diet OR the Mediterranean Diet (MD). If you are in the standard care group (low fat diet) you will be provided with some supermarket vouchers so that you can purchase the food recommended by the dietitian. If you are in the MD group you will be provided with a hamper with examples of the foods you should include in your diet. For more information regarding the dietary components of this study please see the end of this section "dietary intervention'.

It is recommended that you allow approximately 2 hours for this appointment.

Follow Up- telephone
There will be a telephone follow up 2 and 4 weeks after the initial consultation to assess how you are going and to revisit your goals.

Second Appointment
Six weeks after the initial appointment you will be required to return for your second of five face to face appointments. This appointment will be similar to the first appointment where you will be asked to bring all the same questionnaires and food diary along. You will need to fast for a blood test and will undergo a consultation with the dietitian which will involve taking the same measures performed in appointment one. This appointment will not involve any imaging. You should allow approximately 1.0 hours for this appointment

Follow Up- telephone
There will be another telephone follow up on week 9 to assess how you are going and to revisit your goals.

Third Appointment

On week 12 another face to face appointment will be booked in, this will be your third of five appointments. The week 12 appointment will be exactly the same as week 1 including all the tests, imaging, measures and the consultation, with the requirement to fast prior. You should allow approximately 1.5 hours for this consultation.

After the 12 week appointment the intervention stage will be complete and there will be two final reviews to see your progress at 6 and 12 months post commencing the study.

Fourth Appointment
Six months after starting the study you will be asked to return for another face to face appointment. This appointment is a follow up to see how you are going. It will involve bringing in the food diary and all of the questionnaires and fasting for all the tests and measures performed at baseline and 3 months

Fifth Appointment
Twelve months after starting the study you will be asked to attend for a fifth and final face to face appointment. Again all questionnaires will be required and you will be asked to attend fasted.

DEXA Scan Volunteers
If you decide to volunteer to have you body composition analysed throughout the study this will be competed at the first (baseline), third (3 month), and fourth (12month) appointment times. These tests will NOT be carried out at The Alfred and another appointment time will need to be made to attend La Trobe University Bundoora campus. This scan will take about 15 minutes and causes no pain or discomfort. For further information regarding any risks associated with this testing please see question 9 *exposure to ionising radiation*.

Dietary Intervention
As described above you will randomly be assigned to either the standard or intervention arm of this study. The participants assigned to the **standard arm** will be asked to follow a low fat diet which follows the guidelines set out by the Australian Guide to Healthy Eating and the Heart Foundation. These are the guidelines that you would normally be asked to follow by a dietitian for the management of NAFLD. You will be guided to recipes and meal suggestions from the above organisations and will be given resources to guide you with food choices. A supermarket voucher will also be issued to you at three separate time points to fund some of the foods suggested.
Participants who are randomly assigned to the **intervention arm** will be asked to follow a Mediterranean Diet. This differs from the standard recommendations used to treat NAFLD. Participants will be given resources including meal plans and recipes. There will also be a food hamper supplied at three different time points which will provide examples of which foods are being recommended for this diet.
For both groups your food diary will be reviewed at each appointment and the dietitian will work with you to set goals to achieve the dietary guidelines specific to the diet you are working towards.

Results of the study
For participants who are interested there will be a newsletter distributed with the results of the study when the analysis is complete.

Blood and Imaging Results
You will be permitted to discuss blood tests, urine samples and images taken throughout the duration of the study with your research doctor or research dietitian.

Reimbursement
On the days of your appointments for this research project you will be asked to arrive fasted for the purpose of your blood tests. After your blood tests are completed as a reimbursement for your time you will be provided with breakfast. You will also be supplied with either supermarket

vouchers to the value of $20 if you are in the control group or hampers to the value of $80 if you are in the intervention group.

This research project has been designed to ensure that the researchers interpret the results in a fair manner which avoids study doctors or participants jumping to conclusions.

Additional costs
There are no additional costs associated with participating in this research project, nor will you be paid. All tests and medical care required as part of the research project will be provided to you free of charge.

Feedback
After you have completed the three month intervention a researcher will contact you for your feedback. The researcher will arrange a time to ask you some questions over the phone, this will take about 20 minutes. Questions can be sent to you in advance via email so you can think about your responses before the phone call. The aim of these questions is to see how we can improve diet therapy for future research and in the clinical setting.

4 What do I have to do?

Lifestyle
You are still permitted to continue your regular medications as any medications which are not permitted as a part of this study will deem you as ineligible during the screening process.
This study involves recommendations to alter your current diet and this may have some impact on your current lifestyle.

Exercise
While there is no restriction on exercise, if you are not currently exercising your research dietitian may recommend gradually increasing your physical activity.
You are still able to donate blood throughout the duration of the study if you wish to do so.

What restrictions are there for taking part?
All restrictions that will limit participants from taking part will be covered thoroughly in the screening questionnaire.

5 Other relevant information about the research project

This study aims to recruit a total of 94 participants across four hospital sites. All data for this research project will be collected in Australia and all data, except the genetic components which will be collected from blood and cheek swabs, will be analysed at La Trobe University. The researchers working on this project are from La Trobe University, The Alfred Hospital, St Vincent's Hospital, Box Hill Hospital and the Royal Melbourne Hospital. There is also a researcher from University College in Dublin Ireland. All of these researchers are working on this project in collaboration.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with The Alfred Hospital.

7 What are the alternatives to participation?

You do not have to take part in this research project to receive treatment at this hospital. Other options are available; these include asking your doctor to refer you to the hospital dietitian for nutrition therapy. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits for participants in the intervention group, and to a lesser extent in the control arm may include: an improvement in hepatic steatosis (reduced liver fat) and improvement in related symptoms (classified as the Metabolic Syndrome) this may include improved: resistance to insulin and/ or blood pressure. These benefits were seen in patients who followed this diet in a pilot study conducted by Ryan et al 2013.

9 What are the possible risks and disadvantages of taking part?

For female participants
If you do become pregnant whilst participating in the research project, you should advise your study doctor or dietitian immediately. Your study doctor will withdraw you from the research project although no further medical attention should be necessary. However you must not continue in the research if you become pregnant.

If you become upset or distressed during the study
If you become upset or distressed as a result of your participation in the research, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

Risks associated with blood test
Having a blood sample taken may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which tissue is taken could become inflamed. Some people may feel faint when having blood taken, and may occasionally faint. Rarely, there could be a minor infection or bleeding. If this happens, it can be easily treated.

Exposure to ionising radiation
If you choose to take part in this research your participation in a DEXA scan is optional. DEXA scans are a non-invasive, fast and simple procedure.

This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is less than 0.035 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal.

Have you been involved in any other research studies that involve radiation? If so, please tell us. Please keep information contained within the Patient Information and Consent Form about your exposure to radiation in this study, including the radiation dose, for at least five years. You will be required to provide this information to researchers of any future research projects involving exposure to radiation.

10 What will happen to my test samples?

Blood tests and urine samples are required as a mandatory component of this study.

The following tests are routine: HbA1C, HOMR-IR (derived from fasting insulin and glucose), Liver Function Tests: ALT, GGT, AGT, Lipid Profile: Fasting cholesterol (TC, LDL-C, HDL-C & Triglycerides), U&Es, FBE, Fe studies, Vitamin D, blood pressure (SBP/DBP), MR-Spectroscopy ,

The following tests are not routine for your condition but will be required for research purposes: Non Esterified Fatty Acids, , Apo lipoproteins, Cytokines (adiponectin, , hsCRP), Plasma fatty acids, , Serum Cytokeratin 18 Fragments, Peripheral blood mononuclear cells (PBMCs), PMPCM3 (polymorphism), Cheek swabs for DNA analysis, Urine: metabolomics and sodium potassium ratio. DXA (for a sub group).
While the above tests are not taken routinely for your condition they will be taken at the same time as your routine tests, e.g. from the same blood test.

Samples- transfer and storage
Not all blood and urine samples will be analysed immediately after they are taken. These samples will be transferred to La Trobe University where they will be stored in a secure place for a minimum of 7 years for future analysis.
The blood and urine samples will be coded for all participants involved in the study; this means samples will be re-identifiable. Privacy and confidentiality of each sample will be maintained at all times and the re-identifiable list will be saved in password protected documents with passwords accessible strictly to authorised researchers.

Consent for stored samples
This consent forms includes consent to have samples analysed for this research as well as any further analyses approved by an ethics committee and for which are considered to be necessary to increase the knowledge that medical professionals have about this condition.

Tissue Bank
This research does not involve establishment of a tissue bank.

Genetic Testing
While this research will include genetic analysis, this project does not involve genetic testing that would result in information about an identifiable participant's future health risk or having children with a genetic disorder. Nor will this research reveal information that may be relevant to the health of family members who are not part of the project.

11 What if new information arises during this research project?

In the event new information about treatment for NAFLD becomes available during the course of a research project, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some or all of the medications or treatments you have been taking for your condition or for other reasons. It is

important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project. Your study doctor should also explain to you which treatments or medications need to be stopped for the time you are involved in the research project.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team when you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

What happens to the data that has already been collected?
If you do withdraw your consent during the research project, the study doctor, dietitian or relevant study staff will not collect additional personal information or samples from you. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you withdraw from the research project. All samples collected will be retained to complete testing at a later date, if you do not want them to do this, you must tell them before you withdraw from the research study and any samples will be destroyed.

14 Could this research project be stopped unexpectedly?

In the unlikely event that this research project will be stopped unexpectedly there may be a variety of reasons why. These may include reasons such as:
- The dietary therapy is being shown not to be effective
- The diet therapy is being shown to work and not need further testing

15 What happens when the research project ends?

When the research project ends you may continue to follow the diet that has been recommended to you if you choose to.

The results of the study will be provided in a newsletter to those who are interested. Please contact either:

Elena George
E.George@latrobe.edu.au
(03) 9479 5635

Dr Audrey Tierney
A.Tierney@latrobe.edu.au
(03) 9479 5253
If you would like to have a copy of the results sent to you.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

Data and sample retention
All data collected about you will be in a re-identifiable form. This means that it will be labelled with a code that has been assigned to you. The protocol for this code will be saved in a password protected computer document so only researchers who have access to it will be able to identify who each code is assigned to.
All blood and urine samples which have been collected throughout the duration of the study will be stored in a secure place at La Trobe University Bundoora. All questionnaires and computerised data will be stored in a locked cabinet or a password protected computer document. As it is not possible to carry out all the tests for this study some of the blood and urine will be retained and stored for later testing.These samples will be kept for a minimum of seven years (as per the La Trobe University protocol) but this time frame may be longer depending on when funding becomes available for further testing.

Databank
Researchers will retain a databank of information for future related research. This is for analysis that has not necessarily been determined yet and thus not specified. A separate ethics application will be submitted for any such tests.

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information from your health records
Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Publication and presentation of results from this study
It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified.

Your health records
Information about your participation in this research project may be recorded in your health records.

Access to your information and data
In accordance with relevant Australian and Victorian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Confidentiality
Any information obtained for the purpose of this research project and for the future research described in Section 16 that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or in compliance with the law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 Who is organising and funding the research?

This research project is being conducted by La Trobe University in collaboration with The Alfred Hospital. A grant from La Trobe University is funding this project.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of The Alfred Hospital.

All other sites involved will have approval by their associated HREC.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or have any complaints which you wish to put forward please contact the site clinical and complaints contact person listed below.

Clinical contact person

Name	*Dr Audrey Tierney*
Position	*Senior Dietitian*
Telephone	*03 9479 5253*
Email	*A.Tierney@latrobe.edu.au*

Complaints contact person

Name	*Ms Emily Bingle*
Position	*Research Governance Officer at the office of Ethics and Research Governance*
Telephone	*03 9076 3619*
Email	*research@alfred.org.au*

*You will need to tell Ms Bingle the following Alfred Health Number: **76/14**

Consent Form - *Adult providing own consent*

Title	Mediterranean Dietary Intervention study in NAFLD patients
Short Title	MEDINA
Coordinating Principal Investigator/ Principal Investigators	Dr Audrey Tierney A/Prof Catherine Itsiopoulos Professor Stuart Roberts Ms Elena George Ms Anjana Reddy
Location	The Alfred Hospital

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my future care.

I understand that I will be given a signed copy of this document to keep.

I agree to the use of my samples for genetic analysis, as outlined in the relevant Section 10.

I consent to the storage and use of blood and tissue samples taken from me as outlined in Section 10 for use in:

- ☑ this research project
- ☑ other closely related future research
- ☐ any future research

If you wish to take part in the voluntary DEXA scanning component of the study please tick this box ☐ *(Please note this is optional and your decision will not impact your participation in the other parts of the study)*

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Researcher† (please print) _____

Signature _____ Date _____

† An appropriately qualified member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

I understand that, if I decide to discontinue the study treatment, I will not be asked to attend follow-up visits to allow collection of information regarding my health status. Alternatively, a member of the research team may request my permission to obtain access to my medical records for collection of follow-up information for the purposes of research and analysis.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	Mediterranean Dietary Intervention study in NAFLD patients
Short Title	MEDINA
Coordinating Principal Investigator	Dr Audrey Tierney
Principal Investigators	Professor Stuart Roberts A/Prof Catherine Itsiopoulos Ms Elena George Ms Anjana Reddy
Location	The Alfred Hospital

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with The Alfred Hospital or La Trobe University.

☐ I consent to access to my medical records for collection of information on my health status for the purposes of this research.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

3.2 Screening Form

MEDINA STUDY Screening Form		
	Date	☐☐/☐☐/☐☐
	Site Code	☐☐
	Participant Initials	☐☐☐
	Participant Date of Birth	☐☐/☐☐/☐☐
	Participant Code	☐☐☐☐☐☐
	Gender	F ☐ M ☐
	Eligibility Status	Yes ☐ No ☐

Part 1: IMAGING		
Inclusion criteria (must all be marked "yes" for eligibility)* *With the exception of qn. 1.1 which is accepted if 1.2 is marked "yes".		
1.1	Have you undergone an u/s or biopsy for diagnosis of NAFLD in the last 12 months?	Yes ☐
		No ☐

Part 2: NAFLD DIAGNOSIS		
Inclusion criteria (must be marked "yes" for eligibility)		
2.1	Have you been diagnosed with Non Alcoholic Fatty Liver Disease (NAFLD) based on u/s or biopsy findings?	Yes ☐
		No ☐
2.2	Do you have a reading of alanine aminotransferase (ALT) elevated at *(>30 males, >20 females)* and <5 times (ULN)?	Yes ☐

		No ☐

Part 3: INSULIN RESISTANCE		
Inclusion criteria (must be marked "yes" for eligibility)		
3.1	Have you had Fasting glucose tested in the last 12 months? (F) Glucose:_____mmol/L	Yes ☐ No ☐
3.2	Have you had Fasting insulin tested in the last 12 months? (F) Insulin:_____mmol/L	Yes ☐ No ☐
3.3	Do you have a HOMA IR score >2? _____ x _____ / 22.5 = _____units	Yes ☐ No ☐
Part 4: MEDICAL HISTORY		
Exclusion criteria (must all be marked "no" for eligibility)		
4.1	Do you have a HbA1c above 8%?	Yes ☐ No ☐
4.2	Do you have Cardiovascular, Cerebrovascular and/or Peripheral vascular disease?	Yes ☐ No ☐
4.3	Do you have pulmonary, gastrointestinal, renal, metabolic, haematological, neurological, psychiatric, systemic or any acute infectious disease or signs of acute illness?	Yes ☐ No ☐
4.4	Do you have a psychosocial or gastrointestinal malabsorptive condition i.e. coeliac disease?	Yes ☐ No ☐
4.5	Do you currently or have you previously had bulimia nervosa, anorexia nervosa or any other eating disorder?	Yes ☐ No ☐

4.6	Do you currently or have you previously suffered from substance abuse (alcohol or drugs)?	Yes ☐
		No ☐
4.7	Do you have clinical depression or are you currently undergoing psychiatric care?	Yes ☐
		No ☐
4.8	Are you pregnant or breastfeeding?	Yes ☐
		No ☐
4.9	Are you currently on or do you plan on starting a diet in the next 12 months e.g. Light and Easy, Jenny Craig, Optifast etc.?	Yes ☐
		No ☐
4.10	Have you had a change in weight (loss or gain) of more than 5kg in the last 3 months?	Yes ☐
		No ☐

Part 5: MEDICATIONS & SUPPLEMENTS		
Exclusion criteria (must be marked "no" for eligibility)* *With the exception of qn. 4.5 which is accepted if 4.6 is marked "yes".		
5.1	Do you take any immunosuppressant's (e.g. Prednisolone, Imuran, Cytoxan, Sandimmune, Neoral, Copaxone, Methotrexate, CellCept, Rapamune)?	Yes ☐
		No ☐
5.2	Do you take amiodarone and/or perhexiline?	Yes ☐
		No ☐
5.3	Have you started taking OR changed the amount you are taking in the last 3 months of any of the following: Vitamin E, Vitamin C, or Vitamin D more than 3000IU daily?	Yes ☐
		No ☐
5.4	Do you take fish oil or krill oil and have you changed the dose you are taking in the last 3 months?	Yes ☐

Part 6: ALCOHOL CONSUMPTION		
Exclusion criteria (must be marked "no" for eligibility)		
6.1	How many days per week do you consume alcohol? ____/7	Yes ☐ No ☐
6.2	How many <u>standard drinks</u> do you consume on average per day that you consume alcohol: ____standard drinks *Please see attached sheet describing standard drinks.*	Yes ☐ No ☐
6.3	Does this number exceed <u>two</u> standard drinks?	Yes ☐ No ☐

Part 7: ANTHROPOMETRY		
Inclusion criteria (must be marked "yes" for eligibility)		
7.1	Height _____m Weight _____kg BMI _____kg/m^2	
7.2	Is your BMI between 20-40kg/m^2?	Yes ☐ No ☐
7.3	Waist circumference _____cm	

END OF SCREENING FORM

3.3 Personal Information Form

MEDINA STUDY Personal Information Form	**Date**	☐☐/☐☐/☐☐
	Site Code	☐☐
	Participant Initials	☐☐☐
	Participant Date of Birth	☐☐/☐☐/☐☐
	Participant Code	☐☐☐☐☐☐
	Gender	F ☐ M ☐
	Appointment	☐ 0wks ☐ 6wks ☐ 12wks ☐ 6mnth ☐ 12mnth

PERSONAL INFORMATION

Please choose the most relevant option and enter the corresponding number for that option in the box.

Marital Status:
1. Single
2. Married
3. De facto
4. Living with partner
5. Divorced
6. Widowed

☐

Living Arrangements:
1. Living with spouse
2. Living with family
3. Living with friends
4. Living alone

☐

Education Level:
1. Didn't complete secondary school
2. Completed secondary school
3. Apprentice/Trade
4. Certificate/ Diploma
5. Bachelor degree
6. Postgraduate degree (Masters)
7. Postgraduate (Doctoral)

☐

Smoking Status:
1. Smoker

Number of cigarettes per day?
_____

Smoked for _____ years

2. Non Smoker (past smoker)

Quit smoking _____years
_____months ago

3. Non Smoker (never smoked)

☐

Work Status:
1. Employed full time
2. Employed part time
3. Employed casual
4. Unemployed
5. Retired

☐

Occupation:
What is your occupation?

_____

Allocate the percentage of time doing each

Sitting ☐ %

Standing ☐ %

Walking ☐ %

Country Born In:
1. Australia
2. Other (please specify)

_____

Came to Australia _____years ago.

☐

Ethnicity: *(see classifications list at end of document)*
1. Oceanian
2. North West European
3. Southern and Eastern European
4. North African and Middle Eastern
5. South East Asian
6. North East Asian
7. Southern and Central Asian
8. People of the Americas
9. Sub Saharan African

☐

Languages spoken:
1. English

Other(s) *please list all other languages spoken and please circle which language is predominantly spoken at home:*

☐

1 OCEANIAN

- 11 Australian Peoples New Zealand
- 12 Peoples Melanesian and
- 13 Papuan
- 14 Micronesian
- 15 Polynesian

2 NORTH-WEST EUROPEAN

- 21 British
- 22 Irish
- 23 Western European
- 24 Northern European

3 SOUTHERN AND EASTERN EUROPEAN

- 31 Southern European
- 32 South Eastern European
- 33 Eastern European

4 NORTH AFRICAN AND MIDDLE EASTERN

- 41 Arab
- 42 Jewish
- 43 Peoples of the Sudan
- 49 Other North African and Middle Eastern

5 SOUTH-EAST ASIAN

- 51 Mainland South-East Asian
- 52 Maritime South-East Asian

6 NORTH-EAST ASIAN

- 61 Chinese Asian
- 69 Other North-East Asian

7 SOUTHERN AND CENTRAL ASIAN

- 71 Southern Asian
- 72 Central Asian

8 PEOPLE OF THE AMERICAS

- 81 North American
- 82 South American
- 83 Central American
- 84 Caribbean Islander

9 SUB-SAHARAN AFRICAN

- 91 Central and West African
- 92 Southern and East African

MEDINA STUDY Anthropometry and blood pressure record form	**Date**	☐☐/☐☐/☐☐
	Site Code	☐☐
	Participant Initials	☐☐☐
	Participant Date of Birth	☐☐/☐☐/☐☐
	Participant Code	☐☐☐☐☐☐
	Gender	F ☐ M ☐
	Appointment	☐ 0wks ☐ 6wks ☐ 12wks ☐ 6mnth ☐ 12mnth

ANTHROPOMETRY		
Please ensure bladder has been emptied before commencing measurements. Take weight first, followed by blood pressure and then continue with remaining anthropometric measures.		
Height:	Weight:	BMI:
Waist circumference:	Hip circumference:	Waist to Hip Ratio:
Neck circumference:		
Notes		

BIO IMPEDANCE ANALYSIS		
• Ensure the subject has been in a supine position for 4 minutes. • Complete BIA measurement between 4 - 6 minutes.		
Fat mass (kg):	Fat mass (%):	Fat free mass (kg):
Fat mass index (kg/m^2):	Fat free mass index (kg/m^2):	Skeletal muscle mass (kg):
Total body water (L):	Extracellular water (L):	Hydration:
Skeletal muscle mass L-leg (kg):	Skeletal muscle mass R-leg (kg):	
Skeletal muscle mass L-arm (kg):	Skeletal muscle mass R-arm (kg):	Impedance:
Phase angle Φ (50kHz):	BIVA (Ω) Xc (50kHz):	BIVA (Ω) R (50kHz):

BLOOD PRESSURE		
• Take readings AFTER weight has been measured and BEFORE remaining anthropometric measures. • Allow participant to sit for 5 minutes prior to commencing blood pressure measures. • Take at least 2 measures, if systolic or diastolic readings differ by 10mmHg or more continue to take readings until two consecutive measures with less than 10mmHg difference are obtained.		
1.	2.	3.
4.	5.	6.
7.	8.	Heart Rate:

MEDINA STUDY Dietary Consultation Form	**Date**	☐☐/☐☐/☐☐
	Site Code	☐☐
	Participant Initials	☐☐☐
	Participant Date of Birth	☐☐/☐☐/☐☐
	Participant Code	☐☐☐☐☐☐
	Gender	F ☐ M ☐
	Appointment	☐0wks ☐6wks ☐12wks ☐6mnth ☐12mnth

Part 1: MEDICATIONS & SUPPLEMENTS		
This information should be checked at each review for changes. This section should be answered by participant.		
1.1	Do you take any prescription medications?	Yes ☐ *please list below* No ☐
	Medications:	
1.2	Do you take any supplements or non-prescription medications?	Yes ☐ *please list below* No ☐
	Supplements and non-prescription medications:	

Part 2: QUESTIONNAIRES AND FOOD DIARY		
This information should be checked at each appointment and is to be answered by the consulting dietitian. All questions must be answered with 'yes'.		
2.1	Have you validated the 3 day food diary and checked that it includes 2 week days and 1 weekend day?	Yes ☐ No ☐
2.2	Have you checked that all other questionnaires are complete? • PREDIMED checklist (MD or low fat) • Cancer Council FFQ • Active Australia Questionnaire • SF 36	Yes ☐ No ☐

Part 3: APPETITE, BOWELS & SLEEP		
This information should be checked at each appointment and is to be answered by the consulting dietitian.		
3.1	How would you describe your appetite *(qualitative response)?*	
3.2	Do you have regular bowel movements?	Yes ☐ Move to question 3 3 No ☐
3.3	Describe frequency and nature of bowel motions *(qualitative response and please tick if dietitian deems any options relevant)?*	Constipation ☐ Diarrhoea ☐
3.4	How would you describe your sleep patterns *(qualitative response)?*	

Part 4: COMPLETION OF ALL TESTS		
This information should be checked at each review for changes. This section should be answered by participant.		
4.1	Have you had a fasting blood sample taken today?	Yes ☐
		No ☐
4.2	Have you provided a urine sample today?	Yes ☐
		No ☐
4.3	Are you listed to have MR-S imaging? If so have you had your test booked in (baseline, 3mo, 12mo ONLY)? *Record date and time in handbook.*	Yes ☐
		No ☐
		n/a ☐
4.4	Have you elected to have DXA scan as a part of your involvement in this study? (Dietitian to explain if necessary) *Record date and time in handbook.*	Yes ☐
		No ☐

<u>Complete either Part 5A OR 5B depending on which arm participant has been randomised to.</u>

Part 5A: RESOURCES- Intervention arm	
Dietitian to indicate which resources were provided at each appointment.	
Goals sheet (handbook and clinician CRF) *(should be completed at every appointment)*	☐
Mediterranean Diet Food Pyramid	☐
Mediterranean Diet Principals	☐
Food Group Checklist	☐
2 week menu cycle	☐
Shopping List	☐

Mediterranean Diet Label reading	☐
No cooking meal options	☐

Part 5B: RESOURCES- Standard arm	
Dietitian to indicate which resources were provided at each appointment.	
Australian Guide to Healthy Eating	☐
Healthy Eating Guide for weight loss- The Alfred	☐
Low Salt – The Alfred	☐
	☐
	☐

Part 6: GOAL SETTING- Intervention arm
These should be set together with participant. They should be provided with a copy on their resource.
See: Goal Setting for Researcher CRF.

END OF DIETARY CONSULTATION FORM.

Figure S1: Mediterranean Diet: Two week meal plan

WEEK 1

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast	Porridge (cooked rolled oats with skim milk) with honey cinnamon and berries Herbal Tea	2 slices of Soy and Linseed bread with chopped tomato, onion, herbs, olive oil and lemon juice Coffee	Greek style low fat yogurt with chopped fruit, honey, cinnamon and nuts walnuts/ almonds Coffee	2 pieces of Soy and Linseed bread with poached/boiled egg sliced, avocado a squeeze of lemon juice and cracked pepper Herbal Tea	Porridge (cooked rolled oats with skim milk) cinnamon, bananas and honey Herbal Tea	2 slices of Soy and Linseed bread with poached eggs in tomato stew Coffee	Greek style low fat yogurt with chopped fruit, honey and cinnamon. Coffee
Snack	Small handful of Walnuts	Low fat Greek yogurt with honey	Very small handful of Sultanas Sesame snack bar	Low fat Greek yogurt with berries (fresh/frozen) and honey	2 slices of watermelon	Low fat Greek yogurt with honey	2 slices of honeydew
Lunch	Soy and linseed sandwich with spinach, tinned tuna in olive oil, tomato and onion	Left over Lentil soup drizzled with olive oil with 2 slices of soy and linseed bread and a piece of feta Piece of fruit	2 slices of toasted soy and linseed bread with canned sardines in olive oil, with tomato and onion	Left over baked risoni with lamb and salad (lettuce cucumber and spring onion) dressed in olive oil. Slice of Soy and linseed bread	Soy and linseed sandwich with feta cheese and roasted vegetables drizzled with olive oil	Left over Cannellini bean soup (white bean soup) with a slice of grain bread Piece of fruit	Salad (pear, walnut and rocket) dressed with olive oil with tinned salmon and 2 slices of soy and linseed bread Piece of fruit
Snack	Pear	Small handful of Almonds with 2 dried figs	Grapes	3 Dried apricots with a small handful of almonds	Small handful of Walnuts	Small handful of Almonds	Small handful of Walnuts
Dinner	Lentil soup drizzled with olive oil with 1 slice of soy and linseed bread and a piece of feta Glass of wine	Baked snapper with baked potato and boiled greens with olive oil and lemon juice Glass of wine	Baked risoni with lamb and salad (lettuce cucumber and spring onion) dressed in olive oil Glass of wine	Chicken soup with egg and lemon Carrot and coleslaw salad With a slice of soy and linseed bread Glass of wine	Cannellini bean soup (white bean soup) drizzled with olive oil with a slice of soy and linseed bread Glass of wine	Stewed chicken livers* with caramelised red onion in a red wine and oregano sauce, boiled greens with olive oil and lemon juice and with 2 slices of soy linseed bread **can replace chicken livers with chicken fillet* Glass of wine	Green pea casserole with beef Rice pilaf with Greek coleslaw salad Glass of wine
Snack	3 Dry figs	Two slices Rockmelon	Very small handful of Walnuts and a mandarin	Risogalo (rice pudding)	Orange	3 Dried figs	Piece of baklava

WEEK 2

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast	Porridge (cooked rolled oats with skim milk) honey, cinnamon and berries Herbal Tea	2 slices of Soy and Linseed bread with chopped tomato, onion, herbs, olive oil and lemon juice Coffee	Greek style low fat yogurt with chopped fruit, honey, cinnamon and nuts (walnuts/ almonds) Coffee	2 slices of Soy and Linseed bread with ricotta cinnamon and honey Piece fruit Herbal Tea	Porridge (cooked rolled oats with skim milk) honey, cinnamon and bananas Herbal Tea	2 slices of Soy and Linseed bread with poached eggs, spinach and tomato dressed with olive oil and cracked pepper Coffee	Greek style low fat yogurt with chopped fruit, honey and cinnamon Coffee
Snack	Small handful of Walnuts	Low fat Greek yogurt with honey	Very small handful of sultanas Sesame snack bar	Low fat Greek yogurt with berries (fresh or frozen)	2 slices of watermelon	Low fat Greek yogurt with honey sprinkled with walnuts	Slice of honeydew
Lunch	Soy and linseed sandwich with spinach, tinned salmon in olive oil, tomato and onion	Salad (spinach and rocket, tomato, cucumber, capsicum) with tinned 4 bean mix, herbs and feta cheese dressed with olive oil and vinegar 2 slices soy linseed bread Piece of fruit	2 slices of toasted soy and linseed bread with canned sardines, olive oil, tomato and onion	Leftover Beef and cauliflower casserole with salad (lettuce cucumber and spring onion) dressed in olive oil and slice of Soy and Linseed bread	Soy and linseed sandwich with feta cheese and roasted vegetables sprinkled with olive oil	Salad (pear, walnut and rocket) dressed with olive oil with tinned tuna and 2 slices soy linseed bread Piece of fruit	Left over Stuffed tomatoes with rice and bean salad, dressed in olive oil
Snack	Pear	Dried figs with a small handful of almonds	Grapes	Apple and small handful of walnuts	Small handful of Almonds	Apple	Small handful of Walnuts
Dinner	Baked (small) chicken breast*with broccoli and salad both dressed in olive oil With couscous *(recipe not supplied)	Baked salmon with boiled greens and beetroot salad dress in olive oil Baked potato Glass of wine	Beef and cauliflower casserole with salad (lettuce, tomato, cucumber and spring onion) dressed in olive oil and a baked potato Glass of wine	Vegetable bake with Greek salad and feta Glass of wine	Chicken casserole with onions and wine, rice and boiled warm wild greens dressed in olive oil and lemon juice Glass of wine	Stuffed tomatoes with rice and black eyed bean salad, dressed in olive oil Glass of wine	Eggplant moussaka with lamb mince and Greek coleslaw salad 1 slice Soy and Linseed bread Glass of wine
Snack	3 Dry figs	2 slices of Rockmelon	Very small handful of Walnuts with a kiwi fruit	Risogalo (rice pudding)	Pear	3 dried figs and a very small handful of almonds	Piece of baklava

Guidelines to assist you in following the meal plan.

Main meals

- If you do not like one of the meals on the meal plan you may substitute it for another meal which cooks with the same protein, e.g. if you don't like salmon you may have another type of fish or seafood.

Sides

- If you wish to replace a side for something else you must substitute it for the same category of food (vegetables for vegetables, do not swap vegetables for a carbohydrate), e.g. if you don't like broccoli you may instead have another source of leafy greens or vegetables, however you must not replace it for rice.

Left Overs

- Many of the meals make 4-6 serves; these can be frozen to avoid having to cook every time. This especially works well for soups which make many serves and freeze very well. ***OR***
- You may have left over meals from dinner for lunch the following day, ensure that you include the salad/vegetable sides.

Fruit

- Where there is a serve of fruit listed you may choose a serve of any fruit that you enjoy/ have available, e.g. 1 apple, 1 orange, 1 banana, a handful of grapes, a slice of melon etc.

Yogurt

- Where Greek yogurt is listed as a snack you may have it plain or with nuts and honey or with some fresh or dried fruit. Add cinnamon for flavour.

Nuts

- All nuts should be raw and unsalted.
- Almonds and walnuts are preferred but all varieties are acceptable.

Portions

- Some of the meals have recommended portion sizes, these are just a guide. You should eat until you feel satisfied; this may be more or less of the suggested amount of food.

Copyright © 2017 by E.S. George & T. Kucianski
All rights reserved. This resource or any portion thereof may not be reproduced or used in any manner whatsoever without acknowledgment.

LA TROBE UNIVERSITY

Figure S2: Mediterranean Dietary Guidelines – Summary

1. **Use extra virgin olive oil as the main added fat** – *aim for minimum 3-4 tablespoons (60-80ml) per day*

2. **Eat vegetables with every meal** – *include 100g leafy greens, and 200g other vegetables daily; use onion and garlic daily; include 100 g tomatoes daily; fresh or sofrito (tomato based sauce).*

Leafy green veggies 1½ cups (raw) or ¾ cup cooked, 1 medium tomato or one tomato based meal, 2½ cups other veggies (raw) or 1½ cups other veggies (cooked), include onion/garlic in at at least one meal per day (raw or cooked).

3. **Include at least two legumes meals per week** **canned or dry legumes are acceptable, this may include tofu (1 serve = 250 g.* This should replace meat.

One serve is about half a standard can or 1½ cups (cooked).

4. **Eat at least three servings of fish** *(1 serve = 100-150 g)* **or shellfish** *(1 serve = 200g)* **per week** – *include oily fish at least 1 – 2 times per week.*

5. **Eat red meat less often and choose smaller portions** *(150-200g weekly) (beef, lamb, pork).* **Limit white meat** *(200 – 250g per week) (poultry).* **Choose lean varieties.**

*The recommended serving of 'meat' can be eaten in one meal or over multiple, as long as you do not exceed the maximum allowance per week.

Copyright © 2017 by E.S. George & T. Kucianski
All rights reserved. This resource or any portion thereof may not be reproduced or used in any manner whatsoever without acknowledgment.

LA TROBE UNIVERSITY

6. **Eat fresh fruit every day** *(300g or 2 serves).*

*1 serve is equivalent to: 1 large fruit (apple, banana, orange) OR 2 small fruits (kiwi, apricot, plum) OR 2 slices of fruit (watermelon, rockmelon, pineapple) OR 15 grapes or cherries.

7. **Eat a serve of nuts every day** (1 serve = ~30g or 1/3 cup or a small handful daily) **and dried fruit** *(2 tablespoons or 30g)* **as snacks or dessert**;

8. **Eat dairy every day (2 serves) including milk** *(1 serve = 250ml or 1 cup)* **and yoghurt** *preferably Greek style yoghurt (1 serve = 150g or ¾ cup)*;

9. **Eat cheese in moderation**, *about 3 times per week and preferably feta (1 serve = 30g or the size of a matchbox)*;

10. **Include wholegrain breads and cereals with meals** *such as wholegrain bread, rice, pasta and potato (1 serve = 1 slice of bread or ½ cup, 50-60g cooked pasta/rice or 1 small 100g potato)*

11. **OPTIONAL Consume wine in moderation** *(preferably red), (0-2 glasses, 100ml per glass) and always with meals and don't get drunk.*

12. **Have sweets or sweet drinks in moderate amounts** *and on special occasions only.*

13. **Consume no more than 3 eggs per week** *and select free range or omega 3 varieties.*

Copyright © 2017 by E.S. George & T. Kucianski
All rights reserved. This resource or any portion thereof may not be reproduced or used in any manner whatsoever without acknowledgment.

LA TROBE UNIVERSITY

LA TROBE UNIVERSITY

Copyright © 2017 by E.S. George & T. Kucianski
All rights reserved. This resource or any portion thereof may not be reproduced or used in any manner whatsoever without acknowledgment.

Figure S3: MEDITERRANEAN DIET PYRAMID

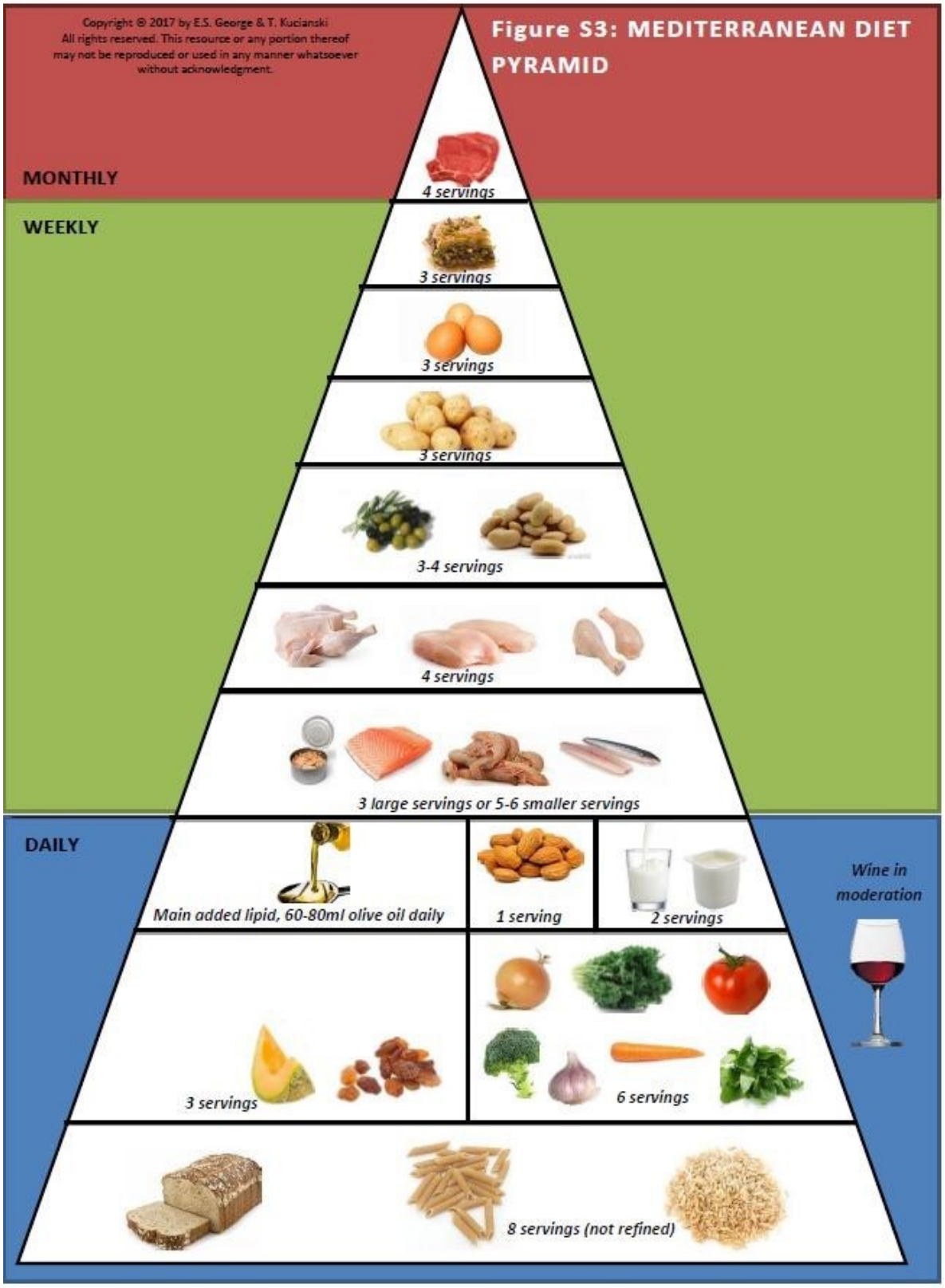
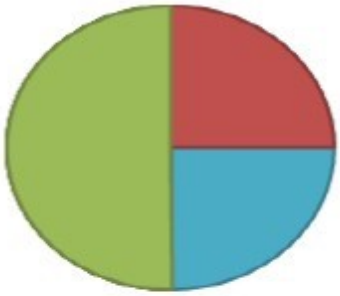


Figure S4: No Cooking Meal options



"No Cooking" Meal Options

Protein

Canned Beans; 4 bean mix, chickpeas, kidney beans, cannellini beans, brown lentils,

Canned Fish; tuna in oil or water, salmon, sardines, trout, mackerel

Canned seafood; oysters, octopus, mussels,

Canned Chicken; in oil or water

Nuts; almonds, walnuts, hazelnuts, whole or spreads

+

Veggies/salad

Salad: Salad mix, Lettuce tomato and cucumber, cabbage and carrot shredded, spinach and tomatoes, beetroot and rocket, celery carrot and capsicum.

Vegetables: Frozen veggies (mixed or individual varieties), left over veggies from dinner.

There are just examples and there are many other possible variations.

+

Carbohydrate

Whole grain crackers

Soy and Linseed bread; plain or toasted

Whole grain wrap; plain or toasted

Rice; Brown rice or brown rice and quinoa sachet can be microwaved, left over from dinner

Couscous; pour enough boiling water to coat grains in a bowl or container, these will be ready to eat in 2 minutes.

Potato (white or sweet); left over from dinner or cooked in the microwave

Pasta; left over from dinner

Noodles; fresh varieties cooked by coating with boiling water for 5 minutes and draining

LA TROBE UNIVERSITY

Copyright © 2017 by E.S. George
All rights reserved. This resource or any portion thereof
may not be reproduced or used in any manner whatsoever
without acknowledgment.

Figure S5: Shopping List

Shopping List

Required Ingredients for the two week menu supplied.

Vegetables

Onions (brown and red)
Garlic
Spinach
Lettuce (cos, rocket)
Cucumber
Spring onions
Rocket
Potatoes
Wild Greens- endive, chicory, dandelion, sow thistle or mixed leafy greens
Eggplant
Frozen peas
Carrots
Cabbage
Capsicum
Green beans
Broccoli
Beetroot (canned ok)
Celery
Zucchini
Cauliflower

Grains

Rolled Oats
Soy and Linseed Bread
Rice
Risoni pasta
Lentils green or brown (dry)
Cannellini beans (dry or canned)
Black eyed beans (dry)
Canned 4 bean mix

Dairy

Skim milk
Greek style Low Fat yogurt
Feta cheese
Reduced fat ricotta

Beverages

Herbal Tea
Red Wine
White wine (for cooking)

Fruit

Frozen berries
Tomatoes
Lemon
Avocado
Pears
Apples
Grapes
Bananas
Melon (honey dew/ rockmelon/ watermelon)
Oranges
Mandarin

Poultry/ Seafood / Meat

Eggs
Tuna tinned in olive oil/spring water
Salmon tinned in olive oil/spring water
Sardines canned
Snapper, whole
Skinned chicken pieces
Lamb loins (lean)
Chicken breast
Salmon
Lamb Mince (lean)
Lean Beef

Other

Olive oil
Spices such as cinnamon, nutmeg, bay leaf, chilli flakes, cloves
Mixed Herbs such as oregano, parsley, thyme
Vinegar- balsamic
Walnuts
Almonds
Sultanas
Dry figs
Honey
Pureed tomatoes
Currents
Sesame snack bars
Breadcrumbs

LA TROBE UNIVERSITY

Copyright © 2017 by E.S. George & T. Kucianski
All rights reserved. This resource or any portion thereof may not be reproduced or used in any manner whatsoever without acknowledgment.

Appendix 5 Alfred Pathology

alfred pathology service

CLINICAL BIOCHEMISTRY METHODS

TEST NAME	METHOD
Cholesterol	Enzymatic
Triglycerides	Glycerol Phosphate Oxidase
Cholesterol, HDL	Accelerator Selective Detergent
Glucose	Hexokinase
High Sensitivity CRP	Immunoturbidimetric
Transferrin	
Albumin	Bromcresol Purple
ALP	Para-nitrophenyl Phospate (p-NPP)
ALT	Enzymatic NADH (without P-5'-P)
AST	
Bilirubin	Diazonium Salt
GT	L-Gamma-glutamyl-3-carboxy-4-nitroanilide Substrate
Total Protein	Biuret
Insulin	Chemiluminescent Microparticle ImmunoAssay
Ferritin	
Iron	Ferene S
Vitamin A	HPLC
Vitamin E	
Haemoglobin A1c	Boranate Affinity HPLC

Assays (excluding HbA1c) are performed on the Abbott Archicentre ci16200 instrument.
HbA1c is performed on a Trinity Premier Hb9210 instrument.

NAME:...Joe D'Agostino..... POSITION:.....Managing Scientist.....

SIGNED:..... DATE:.....19/Sep/17.....

Appendix 6 Questionnaires

6.1 *3-day food diary*

LA TROBE UNIVERSITY

TheAlfred

MEDINA – 3 DAY FOOD DIARY

GENERAL INSTRUCTIONS

- We would like you to keep this diary of everything you eat and drink for 3 complete days, 2 weekdays and 1 weekend day. Try to choose days that represent your typical eating patterns, if you are unable to choose typical days, there is space on the diary in the notes/comments section for you to let us know for e.g. if you went out to dinner or were on holidays.

- This is a **VERY** important part of the study and will greatly add to the information you have already provided us.

- It is very important that you **DO NOT** change what you eat and drink just because you are keeping a record.

- Write any food or drink down as soon as you consume them.

- If you don't eat a particular meal or snack please draw a line through it to let us know that you haven't eaten anything.

- Please use a pen and write clearly.
- If you have any queries please telephone **(03) 9479 5635.**

We thank you for your efforts in keeping this food diary

Study code ID:

INSTRUCTIONS FOR RECORDING FOODS

Please keep an accurate record of everything that you eat and drink. Start with the first thing you eat and drink (when you wake up) and end with the last thing that you eat and drink at bedtime (before you go to bed)

Please keep the following things in mind when you are filling out your food diary;

- **Describe the form** the food is in (canned, powdered, dried, fresh etc).
- Record the **brand name** of the food or any special characteristics e.g. colour of packaging.
- **Describe the food in detail.** Do not simply write peaches, bread or yoghurt..

  E.g. SPC tinned peaches in syrup
  E.g. TIP TOP (toast thick) sliced white bread
  E.g. Yoplait 97% fat free strawberry yoghurt

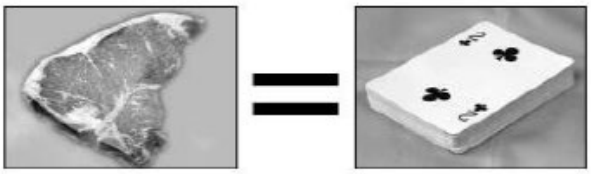
- Include the **quantity** for all foods and beverages. To describe amounts use common household measures (teaspoon, tablespoon, cup), standard serving sizes (a slice of bread, biscuit), metric measurements (cm, grams, mL) or the ruler and circles to measure circumference and thickness **(see page 4).**
- Explain the **cooking method** e.g. Roasted potato or steamed fish.
- Don't forget to **include the fat/oil** that you use to cook with *e.g. 2 tablespoons Olive oil.*
- For **foods with multiple ingredients** e.g. pasta, sandwich, soup, stir-fry, curry etc., please list all ingredients in the dish, including quantities where possible.
- There is space at the back of the food diary to **record recipes.** Feel free to send along a recipe or food label to improve accuracy of your analysis. *Recipes which we have provided you with do not need to be written down.*
- Include **items you add at the table** *e.g. 1 tablespoon Heinz salt reduced tomato sauce, ½ tsp pepper.*
- Many **packet foods have weights** printed on them, so please use these to record how much you ate. If possible, please include the food label.
- When you are **eating out**, and are unable to use household measures use your body for comparison,
  - **Fist** = 1 cup of fruit or 1 medium whole, raw fruit
  - **Thumb** = 30g of cheese or meat
  - **Tip of Thumb** = Approximately 1 teaspoon
  - **One Cupped Hand** = 30-60g of dry goods (nuts, cereal, pretzels etc.)

Study code ID:

Ways to Size Up Your Servings

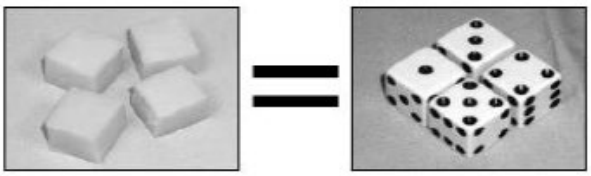
85 grams of meat is about the size and thickness of a deck of playing cards.

85g



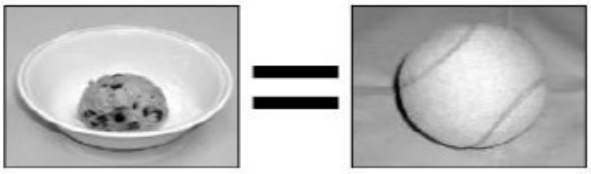
30 grams of cheese is about the size of 4 stacked dice.

30g



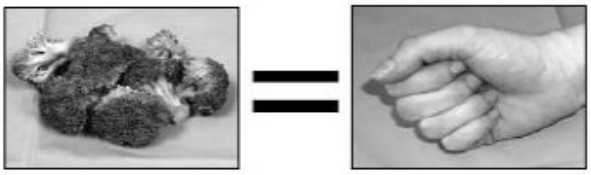
½ cup of ice cream is about the size of a tennis ball

½ Cup



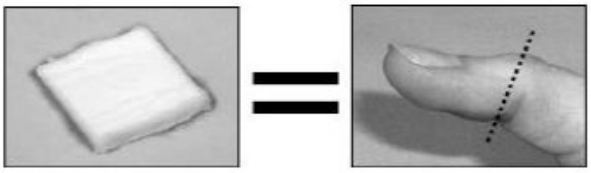
1 cup of mashed potatoes or broccoli is about the size of your fist.

1 Cup



1 teaspoon of butter or peanut butter is about the size of the tip of your thumb.

1 tsp.



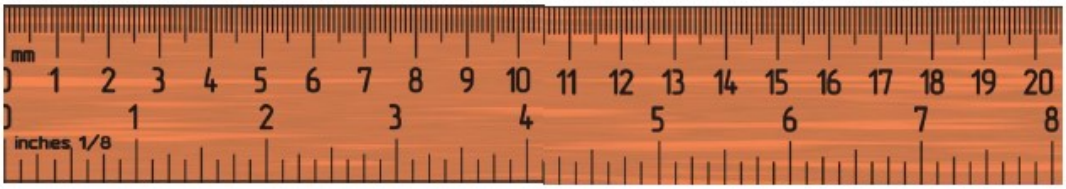
Photos courtesy of: National Dairy Council, USA

Use comparisons- for describing portion sizes, it may be easier to describe portion sizes by comparing to objects eg. Potato the size of a hen's egg, cheese- the size of a matchbox.

Weight on packaging- may also be useful in determining the amount eaten- eg. One buddy size coca cola= 600ml.

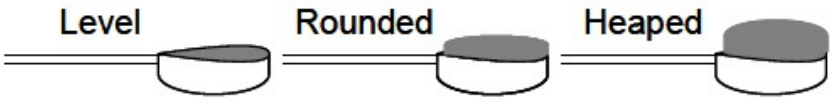
Study code ID:

MEASURING PAGE



How long is it

Please note if level, rounded or heaped, teaspoons or tablespoons.



Study code ID:

FOOD RECORD – EXAMPLE

Day of the week Thursday Date 01 / 09 / 2013

MEAL/SNACK + Time	FOOD and DRINKS consumed (with type and brand)	QUANTITY (cups, spoons, no.)	COOKING METHOD	WHERE/ WHO?
EARLY MORNING 7.00am	Coles low-fat Milk Nestle Milo	1 glass 1 heaped tspn		At home, kitchen table, alone
BREAKFAST 9.00am	Sultana Bran Coles low-fat Milk Tip Top White Bread (Toast sliced) Flora light margarine Black earl grey tea	1 cup ½ cup 2 slices 1 heaped tbspn 1 cup	Toasted	At home, kitchen table, with wife
MORNING TEA 11.00am	Banana Nescafe instant coffee Coles Full Cream Milk Sugar- white	1 med- 15cm long 1 heaped tsp 2 level tbspn 1 level tsp	Raw	At work, desk, alone
LUNCH 1.00pm	White bread roll - Bakers Delight Flora light margarine Virginian Ham Coon Tasty Cheese Tomato Coca Cola Grapes	1 roll- Circle F 2 heaped tspn 3 large slices- 18 cm long, a thick 2 slices (30g) 1 slice- Circle G, b thick 1 Can (375mL) 10 grapes	 Raw Raw	At work, tea room, with colleagues
AFTERNOON TEA				
DINNER 7.00pm	Chicken stir fry **see recipe 1**. White wine - Riesling	1 serve 2 wine glasses		At home, kitchen table, with wife
SUPPER 9.00pm	Chocolate self saucing pudding-white wings Peters vanilla ice cream Natural Almonds	1 large serve- slice 10cm long 5 cm wide 6 cm thick ½ cup ½ cupped hand	Baked	At home, in front of TV, alone
OTHER SNACKS	Tim-Tams Allens snake lollies	3 biscuits hand full		At work with collegues.

Do you feel that today was typical of your usual diet? Yes No Somewhat

Notes/Comments: There was a party at work today and I had the Tim-Tams and lollies

DON'T FORGET TO INCLUDE DRINKS: (Tea, Coffee, Water, Juice, Milk, Alcohol, Soft Drink ETC and QUANTITY- 1 cup/ mug/ 100ml etc) and of you add sugar to coffee tea i.e. 2 sugars, or 1 equal etc.)

Study code ID:

DAY 1

Day of the week _____ Date ____/____/_____

MEAL/SNACK	FOOD and DRINKS consumed (type and brand)	QUANTITY (cups, spoons, no. etc.)	COOKING METHOD	WHERE/ WHO?
EARLY MORNING Time:				
BREAKFAST Time:				
MORNING TEA Time:				
LUNCH Time:				
AFTERNOON TEA Time:				
DINNER Time:				
SUPPER Time:				
OTHER SNACKS				

Do you feel that today was typical of your usual diet? Yes No Somewhat

Notes/Comments:

DON'T FORGET TO INCLUDE DRINKS: (Tea, Coffee, Water, Juice, Milk, Alcohol, Soft Drink ETC and QUANTITY- 1 cup/ mug/ 100ml etc) and of you add sugar to coffee tea i.e. 2 sugars, or 1 equal etc.)

Study code ID:

DAY 2

Day of the week _____ Date ____/____/_____

MEAL/SNACK	FOOD and DRINKS consumed (type and brand)	QUANTITY (cups, spoons, no. etc.)	COOKING METHOD	WHERE/ WHO?
EARLY MORNING Time:				
BREAKFAST Time:				
MORNING TEA Time:				
LUNCH Time:				
AFTERNOON TEA Time:				
DINNER Time:				
SUPPER Time:				
OTHER SNACKS				

Do you feel that today was typical of your usual diet? Yes No Somewhat

Notes/Comments:

DON'T FORGET TO INCLUDE DRINKS: (Tea, Coffee, Water, Juice, Milk, Alcohol, Soft Drink ETC and QUANTITY- 1 cup/ mug/ 100ml etc) and of you add sugar to coffee tea i.e. 2 sugars, or 1 equal etc.)

DAY 3

Day of the week _____ Date ____/____/_____

MEAL/SNACK	FOOD and DRINKS consumed (type and brand)	QUANTITY (cups, spoons, no. etc.)	COOKING METHOD	WHERE/ WHO?
EARLY MORNING Time:				
BREAKFAST Time:				
MORNING TEA Time:				
LUNCH Time:				
AFTERNOON TEA Time:				
DINNER Time:				
SUPPER Time:				
OTHER SNACKS				

Do you feel that today was typical of your usual diet? Yes No Somewhat

Notes/Comments:

DON'T FORGET TO INCLUDE DRINKS: (Tea, Coffee, Water, Juice, Milk, Alcohol, Soft Drink ETC and QUANTITY- 1 cup/ mug/ 100ml etc) and of you add sugar to coffee tea i.e. 2 sugars, or 1 equal etc.)

Study code ID:

RECIPES

EXAMPLE

Recipe: Chicken Stir-fry

Number of servings this recipe makes: 4 **Number of servings you ate:** 1

INGREDIENTS	AMOUNT	COOKING METHOD
Chicken thighs	500g	Chicken and vegetables fried in oil. Sauce added and peanuts sprinkled on top. Rice cooked using rice cooker
Canola oil	2 tablespoons	
Carrot	1 med – 18cm long	
Red capsicum	1 med – circle H, 11cm high	
Snow peas	100g	
Peanuts (salted)	1 handful	
Kantong Sweet n Sour sauce	1 jar (300g)	
Basmati rice	2 cup uncooked	

Recipe: _____

Number of servings this recipe makes:_____ **Number of servings you ate:**_____

INGREDIENTS	AMOUNT	COOKING METHOD

Recipe: _____

Study code ID:

Number of servings this recipe makes:_____ Number of servings you ate:_____

INGREDIENTS	AMOUNT	COOKING METHOD

Recipe: _____

Number of servings this recipe makes:_____ Number of servings you ate:_____

INGREDIENTS	AMOUNT	COOKING METHOD

Recipe: _____

Number of servings this recipe makes:_____ Number of servings you ate:_____

INGREDIENTS	AMOUNT	COOKING METHOD

Recipe: _____

Number of servings this recipe makes:_____ Number of servings you ate:_____

INGREDIENTS	AMOUNT	COOKING METHOD

6.2 PREDIMED Mediterranean Diet Checklist

PREDIMED Checklist

Questions	Criteria for 1 point	Give: 1 or 0
1. Do you use olive oil as main culinary fat?	Yes	
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out of house meals etc)?	≥4 tbsp	
3. How many vegetable servings do you consume per day? [I serving: 200g (consider side dished as a half serving)]	≥2 (≥1 portion raw or as salad)	
4. How many fruit units (including natural fruit juices) do you consume per day?	≥3	
5. How many serving of red meat, hamburger or meat products (ham sausage etc.) do you consume per day	<1	
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12g)	<1	
7. How many sweetened and/ or carbonated beverages do you drink per day?	<1	
8. How much wine do you drink per week?	≥7 glasses	
9. How many servings of legumes do you consume per week? (1 serving : 150g)	≥3	
10. How many servings of fish or shellfish do you consume per week? (1 serving 100-150g of fish or 4-5 units or 200g shellfish)	≥3	
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits or custard?	<3	
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30g)	≥1	
13. Do you preferentially consume chicken, turkey or rabbit meat instead or veal, pork, hamburger or sausage?	Yes	
14. How many times per week do you consume vegetables, pasta or other dishes seasoned with sofrito (sauce made with tomato and onion, leek or garlic and simmered with olive oil)?	≥2	
TOTAL SCORE OUT OF 14		

PREDIMED Checklist

Questions	Criteria for 1 point	Give: 1 or 0
15. How much olive oil do you consume in a given day (including oil used for frying, salads, out of house meals etc)?	2 or less tablespoons (1 tablespoon = 10ml)	
16. Do you remove visible fat (or the skin) from chicken, duck, pork, lamb or veal meats before cooking and the fat of soups, broths, and cooked meat dishes before consumption?	Yes	
17. How many servings of fat-rich meats, hamburger, commercial ground meat, sausage, cold meat, cured ham, bacon, salami or offal do you consume per week? (meat serving: 100g; salami or bacon: 30g)	1 or less	
18. How many servings of butter, margarine, lard, mayonnaise, milk cream or milk based ice cream do you consume per week? (spread fat: 12g; ice cream: 100g)	1 or less	
19. Do you exclusively consume low fat dairy products?	Yes (id. If no dairy consumption)	
20. How many times per week do you prepare rice, pasta, potato or legume dishes by using 'sofrito' sauce (based on olive oil), bacon, salami, or fatty meats such as pork or lamb ribs?	2 or less	
21. How many time per week do you consume fatty fish or seafood canned in oil?	1 or less	
22. How many servings of commercial sweets or industrial bakery products (not homemade), such as cakes, cookies, biscuits or custard do you consume per week? (cake serving: 80g; biscuits 40g)	1 or less	
23. How many times per week do you consume nuts (including peanuts), potato chips, French fries, or commercial snacks?	1 or less	
TOTAL SCORE OUT OF 9		

Appendix 7 The serve sizes for the Xyris, Foodworks, Food Groups

The following is a direct extract from the Foodworks website;
https://foodworks.zendesk.com/hc/en-us/articles/205716789-What-are-the-serve-sizes-for-the-Xyris-food-groups-

February 18, 2016 12:13

The serve sizes for the Xyris food groups are similar to those used in the *Australian Guide to Healthy Eating (AGHE)*. They are based on serves sizes either in the technical document supporting the AGHE, *A modelling system to inform the revision to the Australian Guide to Healthy Eating* or in the *Australian Dietary Guidelines Summary*.

In the Xyris system, for each food group, a single food has been used as a reference food, e.g. a slice of mixed grain bread for the *whole grains* group, then serve sizes have been produced for the rest of the group. To assign serve sizes for the rest of the group, sometimes a nutrient proxy has been used. For example, in the *whole grains* group, the serve size is based on the amount of starch in a slice of mixed grain bread.

The following table defines the serve sizes used for the Xyris food groups.

GROUP AND SUBGROUP	SERVE SIZE	NOTES	BASED ON
Grains			
Refined grains	16g starch	Equivalent starch content to 40g of *Bread, from white flour, commercial* (AUSNUT 2011-13)	*A modelling system* – p38
Whole grains	14g starch	Equivalent starch content to 40g of *Bread, mixed, grain, commercial* (AUSNUT 2011-13)	*A modelling system* – p38
Fruit			
Citrus, melons and berries	Fresh: 350kJ		Fresh:

Other fruit	Dried: 30g		*A modelling system* – p38 Dried: *Australian Dietary Guidelines Summary* – p17
Fruit juice	125mL	Assumed specific gravity: 1.05g/mL	*Australian Dietary Guidelines Summary* – p17
Vegetables			
Dark green vegetables	100kJ		*A modelling system* – p38 'Green & brassica' vegetables
Red and orange vegetables			
- Tomatoes	150kJ		*A modelling system* – p38 'Orange' vegetables
- Other red and orange vegetables			
Starchy vegetables			
- Potatoes	250kJ		*A modelling system* – p38 'Starchy vegetables'
- Other starchy vegetables			
Legumes (counted as vegetables)	350kJ		
Other vegetables	100kJ		
Protein foods			
Red meats	20g protein	Equivalent protein content to 65g of *Beef, blade steak, fully-trimmed, baked, roasted, fried, grilled or bbq'd, no added fat* (AUSNUT 2011-13)	*A modelling system* – p38 *Australian Dietary Guidelines Summary* – p22 'cooked lean red meats'
Poultry	23g protein	Equivalent protein content to 80g of *Chicken, breast,*	*Australian Dietary Guidelines Summary*

		flesh, baked or roasted, no added fat (AUSNUT 2011-13)	– p22 *'cooked lean poultry'*
Eggs	13g protein	Equivalent protein content to 100g (edible portion) of *Raw chicken eggs* (AUSNUT 2011-13)	*A modelling system* – p38
Processed meats	20g protein	Equivalent protein content to 65g of *Beef, blade steak, fully-trimmed, baked, roasted, fried, grilled or bbq'd, no added fat* (AUSNUT 2011-13)	*A modelling system* – p38 *Australian Dietary Guidelines Summary* – p22 'cooked lean red meats'
Organ meats			
Seafood **high** in long-chain n-3 fatty acids	23g protein	Equivalent protein content to 100g of *Barrumundi, boiled, microwaved, steamed or poached, no added fat* (AUSNUT 2011-13)	*Australian Dietary Guidelines Summary* – p22 'cooked fish fillet'
Seafood **low** in long-chain n-3 fatty acids			
Nuts and seeds	30g		*A modelling system* – p38
Legumes (counted as protein foods)	9g protein	Equivalent protein content to 170g of *Tofu (soy bean curd), silken or soft, as purchased* (AUSNUT 2011-13)	*Australian Dietary Guidelines Summary* – p22 'tofu'
Soy products			
Dairy			
Milk	300mg calcium	Equivalent calcium content to 250mL of *Milk,cow,fluid, reduced fat (1%)* (AUSNUT 2011-13)	*Australian Dietary Guidelines Summary* – p25 '1 cup (250 mL)'
Cheese			
Yoghurt			
Milk alternatives			
Oil equivalents	4.6g (1 teaspoon)	Equivalent to 1 teaspoon of *Oil,*	

		olive (AUSNUT 2011-13)	
Solid fat equivalents	4.8g (1 teaspoon)	Equivalent to 1 teaspoon of *Butter, plain, no added salt* (AUSNUT 2011-13)	
Added sugars	4.2g (1 teaspoon)	Equivalent to 1 teaspoon of *sugar, white, granulated or lump* (AUSNUT 2011-13)	
Alcoholic drinks	10g alcohol (1 standard drink)		
Water	mL		

Appendix 8 Ethics Approval Certificates

8.1 *La Trobe University*

University Human Ethics Committee

RESEARCH SERVICES

MEMORANDUM

To: **Dr Audrey Tierney, Department of Dietetics and Human Nutrition, FHS**
Elena Papamiltiadous, Department of Dietetics and Human Nutrition, FHS

From: **Executive Officer, La Trobe University Human Ethics Committee**

Subject: **UHEC acceptance of The Alfred HREC approved project – 76/14**

Title: **MEDINA: Mediterranean Dietary Intervention in NAFLD Patients**

Date: **2 May 2014**

Thank you for submitting the above protocol to the University Human Ethics Committee (UHEC). Your material was forwarded to the UHEC Chair for consideration. Following evidence of a full review and subsequent final approval by the **The Alfred HREC**, the UHEC Chair agrees that the protocol complies with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and is in accordance with La Trobe University's *Human Research Ethics Guidelines*.

Endorsement is given for you to take part in this study in line with the conditions of final approval outlined by **The Alfred HREC.**

Limit of Approval. La Trobe UHEC endorsement is limited strictly to the research protocol as approved by **The Alfred HREC.**

Variation to Project. As a consequence of the previous condition, any subsequent modifications approved by **The Alfred HREC** for the project should be notified formally to the UHEC.

Annual Progress Reports. Copies of all progress reports submitted to **The Alfred HREC** must be forwarded to the UHEC. Failure to submit a progress report will mean that endorsement for your involvement this project will be rescinded. An audit related to your involvement in the study may be conducted by the UHEC at any time.

Final Report. A copy of the final report is to be forwarded to the UHEC within one month of it being submitted to **The Alfred HREC.**

If you have any queries on the information above please e-mail: **humanethics@latrobe.edu.au** or contact me by phone.

On behalf of the La Trobe University Human Ethics Committee, best wishes with your research!

Kind regards,

Sara Paradowski
Executive Officer – Human Ethics / University Human Ethics Committee
Research Integrity Unit / Research Services
La Trobe University Bundoora, Victoria 3086
P: (03) 9479 – 1443 / F: (03) 9479 - 1464
http://www.latrobe.edu.au/researchers/starting-your-research/human-ethics

8.2 Alfred Health

TheAlfred

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 76/14

Project Title: MEDINA: Mediterranean Dietary Intervention in NAFLD Patients

Principal Researcher: Ms Audrey Tierney

Project Proposal Version 2 **dated:** 6-Mar-2014

Participant Information and Consent Form Version 4 **dated:** 31-Mar-2014

*was considered by the Ethics Committee on **27-Mar-2014**, meets the requirements of the National Statement on Ethical Conduct in Human Research (2007) and was **APPROVED** on **17-Apr-2014***

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or progress report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- Any expiry of the insurance coverage provided with respect to sponsored clinical trials and proof of re-insurance;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

- A Progress Report on the anniversary of approval and on completion of the project *(forms to be provided)*;

The Ethics Committee may conduct an audit at any time.

All research subject to the Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Hospital Ethics Committee is a properly constituted Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research (2007).

SPECIAL CONDITIONS

None

SIGNED:

R Frew
Secretary, Ethics Committee

Please quote project number and title in all correspondence

easternhealth
GREAT HEALTH AND WELLBEING

Office of Research and Ethics
T 03 9895 3398
F 03 9094 9610
E ethics@easternhealth.org.au
W easternhealth.org.au/research-ethics
ABN 68 223 819 017
www.easternhealth.org.au

Human Research Ethics Committee
AUTHORISING Ethical Approval – Granted

28 July 2015

Dear Dr Audrey Tierney

LR31/2015 - MEDINA: Mediterranean Dietary Intervention Study In Non Alcoholic Fatty Liver Disease

---

Principal Investigators: Dr Audrey Tierney, A/Professor Amanda Nicoll and Ms Elena Papamiltiadous
Student Investigator: Ms Tonya Paris
Eastern Health Sites: Box Hill and Maroondah Hospital

Approval Period: On-going - subject to a satisfactory progress report being submitted annually

Thank you for the submission of the above project for review. The project has been reviewed and approved by The Alfred HREC. Oversight by Eastern Health Human Research Ethics Committee has been completed and **AUTHORISATION** to commence research is granted for the recruitment of Eastern Health patients at Box Hill H LR72-2015 Final Approval 27 July 15ospital and Maroondah Hospital.

Documents submitted for review:

- Low Risk & Negligible Risk Research Application Form version 1 dated 8 April 2015
- CVs from researchers Audrey Tierney, Elena Papamiltiadous and Tonya Paris
- Cover Letter Ethics Application 9 April 2015
- Alfred Participants information consent form version 8 dated 3 July 2015
- Research Proposal version 5
- NEAF version 2 dated 3 March 2014
- Confidentiality agreements from researchers Tierney, Papamiltiadous and Paris
- Research Proposal Version 5
- Budget proposal
- Honours Project 2015
- Alfred HREC Certificates of approval of amendment dated 17 April 2014, 23 June 2014, 9 October 2014, 26 February 2015, 11 March 2015 and 14 July 2015

Reporting Requirements:

Please note, an annual progress report is required every February for the preceding calendar year until project completion. Continuing approval is subject to the timely submission of a satisfactory progress report. Progress report template can be downloaded from our web-page:
http://www.easternhealth.org.au/research-ethics/research-ethics/quick-links-to-forms-and-templates

Please ensure you notify the Ethics Committee of all personnel changes and any serious adverse events that may affect study conduct. Any changes to the approved Protocol or other approved documents must be submitted for ethical review and approval prior to use.

Please quote our reference number **LR31/2015** in all future correspondence.

Yours sincerely

Jacob Achuoth
Research Ethics and Governance Officer
Office of Research and Ethics

On behalf of:

1. Eastern Health Human Research Ethics Committee (Ethics Approval)
2. Executive Director Medical Services and Research (Site Authorisation)

Copy to: **A/Professor Amanda Nicoll, Ms Elena Papamiltiadou and Ms Tonya Paris**

Confidentiality, Privacy & Research

Research data stored on personal computers, USBs and other portable electronic devices must not be identifiable. No patients' names or UR numbers must be stored on these devices.

Electronic storage devices must be password protected or encrypted.

The conduct of research must be compliant with the conditions of ethics approval and Eastern Health policies.

Publications

Whilst the Eastern Health Human Research Ethics Committee is an independent committee, the committee and Eastern Health management encourage the publication of the results of research in a discipline appropriate manner. Publications provide evidence of the contribution that participants, researchers and funding sources make.

It is very important that the role of Eastern Health is acknowledged in publications.

PO Royal Melbourne Hospital
Parkville Victoria 3050
Telephone 61 3 9342 8530
Facsimile 61 3 9342 8548
Email: research@mh.org.au
Website: http://www.thermh.org.au/research/researchers
ABN 73 802 708 972

OFFICE FOR RESEARCH

MELBOURNE HEALTH

MELBOURNE HEALTH HUMAN RESEARCH ETHICS COMMITTEE

ETHICAL APPROVAL OF A RESEARCH PROJECT

Dr Siddharth Sood
Melbourne Health
Level 3 Centre
Department of Gastroenterology
Pakville
3050

03 September 2015

Dear Dr Siddharth Sood,

AU RED HREC Reference Number: HREC/15/MH/283

MH Project Number: 2015.157 (MAP study)

Project Title: MEDINA: Mediterranean Dietary Intervention in NAFLD Patients

I am pleased to advise that the above project has received ethical approval from the Melbourne Health HREC. The Melbourne Health HREC is accredited by the Consultative Council for Human Research Ethics under the single ethical review system.

HREC Approval Date: 02 September 2014

Participating Sites:

- Royal Melbourne Hospital

Approved Documents:

- Protocol, Version 5.
- The Alfred Hospital Master Participant Information and Consent Form, Version 8, dated 03 July 2015.
- Diagnostic Medical Physicist's Assessment - RSO Letter (Latrobe University)
- SF-36 – Your Health and Wellbeing Questionnaire.
- The Active Australia Survey
- Participant Handbook – Low Fat Diet
- Participant Handbook – Mediterranean Diet
- MEDINA STUDY Screening Form
- MEDINA STUDY Personal Information Form
- MEDINA STUDY Anthropometry and Blood Pressure Record Form
- Chart - Event and location for RMH Patients, Version 1.

Site Specific Assessment:

Site
You are now required to submit this HREC Approval letter with an electronic copy of the approved documents named above as part of the Site Specific Assessment application to the Research Governance Officer at each site of the above listed participating sites, to obtain approval to commence the project at each site.

Conditions of Ethics Approval:

In order to comply with the National Statement on Ethical Conduct in Human Research 2007, Guidelines for Good Clinical Research Practice and Melbourne Health Research Policies and Guidelines you are required to:

- Submit a copy of this letter (via the principal investigator at each site) to the person responsible for radiation safety at each participating site – ***do this if*** the project involves exposure to ionising radiation and the Radiation Safety Officer (RSO) / Medical Physicist for that site has advised that the project needs to be added to the site's Licence for Research Involving Human Volunteers issued by the Department of Health Radiation Safety Section. (See information re radiation requirements at www.health.vic.gov.au/cchre). *Note:* A project cannot commence at a site until the Principal Investigator at that site has received notification from his/her RSO that the project has been added to that site's licence;
- Submit to the HREC for approval any proposed amendments to the project including any proposed changes to the Protocol, Participant Information and Consent Form/s and the Investigator Brochure;
- Notify the reviewing HREC of any adverse events that have a material impact on the conduct of the research in accordance with the NHMRC Position Statement: Monitoring and reporting of safety for clinical trials involving therapeutic products May 2009;
- Notify the HREC of any unforseen events;
- Notify the HREC of your inability to continue as Principal Investigator or any other change in research personnel involved in the project;
- Notify the HREC if a decision is taken to end the study at any of the Victorian sites prior to the expected date of completion or failure to commence the study within 12 months of the HREC approval date at any of the Victorian sites;
- Notify the HREC of any other matters which may impact the conduct of the project.
- If the study is a clinical trial, Melbourne Health requires registration of clinical trials in a public trials registry at or before the time of first patient enrolment as a condition of consideration for publication, in accordance with ICMJE http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

Reporting

You are required to submit to the HREC:

- An Annual Progress Report every 12 months (or more frequently as requested by the reviewing HREC) for the duration of the project. This report is due on the anniversary of HREC approval. Continuation of ethics approval is contingent on submission of an annual report in a timely manner; and
- A comprehensive Final Report upon completion of the project.

The HREC may conduct an audit of the project at any time.

Please Note: Templates for reporting Amendments, Adverse Events, Annual Report/Final Reports, etc. can be accessed from: www.health.vic.gov.au/cchre

Please refer to the Melbourne Health Office for Research website to access guidelines and other information and news concerning research at Melbourne Health:
https://www.thermh.org.au/research/researchers

A list of those HREC members present at the review of this project can be obtained from the above website.

Yours sincerely

Ms Jessica Turner
Manager - Human Research Ethics Committee

Appendix 9. Power calculations for inflammatory markers (Chapter 4)

HS-CRP

t tests - Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.5115464
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8251073
	Critical t	=	1.9799304
	Df	=	120
	Sample size group 1	=	61
	Sample size group 2	=	61
	Total sample size	=	122
	Actual power	=	0.8002373

t tests - Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.5579283
	α err prob	=	0.05
	Sample size group 1	=	19
	Sample size group 2	=	14
Output:	Noncentrality parameter δ	=	1.5840258
	Critical t	=	2.0395134
	Df	=	31
	Power (1-β err prob)	=	0.3356851

TNF-alpha

t tests - Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.4755703
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8335371
	Critical t	=	1.9770537
	Df	=	140
	Sample size group 1	=	71
	Sample size group 2	=	71
	Total sample size	=	142
	Actual power	=	0.8034719

t tests - Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.2130879

	α err prob	=	0.05
	Sample size group 1	=	21
	Sample size group 2	=	18
Output:	Noncentrality parameter δ	=	0.6633953
	Critical t	=	2.0261925
	Df	=	37
	Power (1−β err prob)	=	0.0990598

IL-6

t tests − Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.3807930
	α err prob	=	0.05
	Power (1−β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8240365
	Critical t	=	1.9709056
	Df	=	218
	Sample size group 1	=	110
	Sample size group 2	=	110
	Total sample size	=	220
	Actual power	=	0.8027858

t tests − Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.2501374
	α err prob	=	0.05
	Sample size group 1	=	21
	Sample size group 2	=	18
Output:	Noncentrality parameter δ	=	0.7787395
	Critical t	=	2.0261925
	Df	=	37
	Power (1−β err prob)	=	0.1180986

ADIPONECTIN

t tests − Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.2953081
	α err prob	=	0.05
	Power (1−β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8093099
	Critical t	=	1.9665755
	Df	=	360
	Sample size group 1	=	181
	Sample size group 2	=	181
	Total sample size	=	362
	Actual power	=	0.8000633

t tests - Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.1453448
	α err prob	=	0.05
	Sample size group 1	=	21
	Sample size group 2	=	18
Output:	Noncentrality parameter δ	=	0.4524943
	Critical t	=	2.0261925
	Df	=	37
	Power (1-β err prob)	=	0.0725475

LEPTIN

t tests - Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.4336886
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8273038
	Critical t	=	1.9741852
	Df	=	168
	Sample size group 1	=	85
	Sample size group 2	=	85
	Total sample size	=	170
	Actual power	=	0.8026558

t tests - Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.2601148
	α err prob	=	0.05
	Sample size group 1	=	21
	Sample size group 2	=	18
Output:	Noncentrality parameter δ	=	0.8098017
	Critical t	=	2.0261925
	Df	=	37
	Power (1-β err prob)	=	0.1237844

RESISTIN

t tests - Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size d	=	0.2245772
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1

Output:	Noncentrality parameter δ	=	2.8094599
	Critical t	=	1.9637730
	Df	=	624
	Sample size group 1	=	313
	Sample size group 2	=	313
	Total sample size	=	626
	Actual power	=	0.8009928

t tests - Means: Difference between two independent means (two groups)

Analysis:	Post hoc: Compute achieved power		
Input:	Tail(s)	=	Two
	Effect size d	=	0.0515504
	α err prob	=	0.05
	Sample size group 1	=	21
	Sample size group 2	=	18
Output:	Noncentrality parameter δ	=	0.1604891
	Critical t	=	2.0261925
	Df	=	37
	Power (1-β err prob)	=	0.0528056

Appendix 10

Consort Checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	164
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	164
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	165-169
	2b	Specific objectives or hypotheses	169-170
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	171
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	171
	4b	Settings and locations where the data were collected	171
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	172-173
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	173-176
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a
Sample size	7a	How sample size was determined	179-180
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	172
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	172
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	172
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	172
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	172

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	176-179
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	176-179
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	176-179
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 4.1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 4.1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	180
	14b	Why the trial ended or was stopped	180
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Page 180 and tables 4.1 – 4.3
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Tables 4.1-4.4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Tables 4.1-4.4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n/a
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Section 2 of this chapter (4.8 onward)
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	n/a
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	256-257
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	256-257
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	248-258
Other information			
Registration	23	Registration number and name of trial registry	171
Protocol	24	Where the full trial protocol can be accessed, if available	171
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	7

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Appendix 11

Supplementary Materials

Supplementary Table 1. *Associations of Anthropometric Measurements and Biochemical Markers with Inflammatory cytokines and adipokines in a NAFLD cohort*

Visceral Fat (L)	**0.22**	**0.16**	**-0.13**	**0.07**	**0.05**	**0.26**
Heart Rate	0.28	0.20	0.14	-0.30	0.26	-0.05
GGT	0.11	-0.02	0.07	0.04	0.05	0.11
ALP	0.00	0.23	-0.03	0.20	0.14	-0.04
Bilirubin	-0.05	-0.02	0.09	-0.19	-0.29	0.27
Cholesterol	0.11	-0.21	-0.13	0.17	0.18	-0.13
LDL	0.05	-0.23	-0.07	0.05	0.08	-0.05
Triglycerides	0.00	-0.02	-0.11	0.06	0.10	-0.03
Iron	-0.13	-0.05	-0.14	-0.07	-0.14	0.27
Transferrin	0.00	-0.21	0.02	0.21	0.16	-0.06
Transferrin Sat	-0.13	0.07	-0.07	-0.11	-0.12	0.21

Supplementary Table 2. *Associations of Nutrients and Food Group Intake with Markers of Inflammation in a NAFLD cohort*

	Correlation Coefficients					
	hs-CRP	**TNF-α**	**IL-6**	**Adiponectin**	**Leptin**	**Resistin**
Macronutrients						
Protein (% of total E)	0.08	-0.13	-0.16	0.04	-0.15	0.22
Carbohydrate (% of total E)	0.08	-0.18	0.06	-0.04	0.06	-0.23
Sugars (g)	-0.15	-0.08	0.18	0.03	0.01	-0.16
Sugars (% of total E)	0.27	0.06	-0.10	0.27	0.27	0.18
Saturated Fat (g)	-0.31	0.28	0.25	-0.07	-0.09	-0.08
Saturated fat (% of total fat)	0.22	0.11	0.26	0.18	0.26	0.15
Mono-unsaturated fat (% of total fat)	0.17	0.20	0.01	-0.17	-0.11	0.11
Dietary Fibre (g)	-0.25	-0.04	-0.04	-0.03	-0.21	-0.18
Alcohol (g)	0.30	0.19	-0.08	0.04	-0.09	-0.06
DPA (g)	-0.12	0.28	0.15	0.09	0.09	0.01
DHA (g)	-0.21	0.26	0.06	-0.18	0.01	-0.27
Trans Fatty Acids (g)	-0.15	0.30	0.26	0.10	0.05	-0.01
Vitamin C (mg)	0.19	-0.09	0.12	0.07	0.01	-0.07
Sodium (mg)	-0.19	0.28	0.12	-0.23	-0.20	0.13
Potassium (mg)	-0.16	-0.01	0.02	-0.03	-0.25	-0.06
Iron (mg)	-0.21	-0.03	0.08	-0.14	-0.28	-0.17
Wholegrains	-0.28	-0.09	-0.15	0.22	-0.05	-0.16
Vegetables	0.03	0.04	0.13	0.20	-0.01	0.05
Fruit	-0.03	-0.08	-0.16	-0.03	-0.03	-0.07
Red meats	-0.03	0.21	0.15	0.03	0.05	0.18
Eggs	-0.29	0.02	0.16	-0.03	0.07	0.02
High long chain omega-3	-0.02	0.10	0.09	-0.12	-0.09	-0.22

Low long chain omega-3	-0.06	0.16	-0.06	-0.15	0.05	-0.11
Legumes (CHO)	-0.11	0.14	0.19	0.20	0.30	0.10
Dairy	-0.08	-0.23	0.11	0.14	-0.18	0.09
Milk	-0.15	-0.26	-0.05	-0.06	-0.26	0.04
Yoghurt	0.19	0.02	0.22	0.12	0.28	-0.07
Cheese	-0.06	-0.10	-0.03	0.20	-0.09	0.05
Solid fat equivalents	-0.29	0.28	0.29	-0.03	0.02	0.02
Added Sugars	-0.08	-0.03	0.20	0.07	0.14	-0.07
Alcoholic beverages	0.30	0.19	-0.08	0.04	-0.09	-0.06

Supplementary Table 3. *Effects of Dietary Intervention on Anthropometry, Body Composition and Haemodynamic measures*

Characteristics	**Total**			**Control (LFD)**			**Intervention (MedDiet)**			**P[a]**	**P[b]**			
	n	mean	SD	n	mean	SD	n	mean	SD		**Control**	**P[c]**	**Intervention**	**P[d]**
Anthropometry														
NC (cm)														
Baseline	42	39.27	4.94	23	40.14	4.83	19	38.22	5.00	0.214	0.073		0.829	
Mid-Intervention	36	39.01	4.58	18	39.24	4.75	18	38.78	4.53	0.765		1.000		1.000
End-Intervention	39	40.01	7.58	21	41.00	9.48	18	38.85	4.47	0.383		1.000		1.000
Change		0.53	1.01		0.60	1.77		0.45	0.79	0.943				
HC (cm)														
Baseline	41	109.60	15.12	22	109.83	16.07	19	109.33	14.36	0.875				
Mid-Intervention	36	110.83	16.61	18	112.15	17.33	18	109.50	16.25	0.696	0.637	0.492	0.946	0.76
End-Intervention	39	108.55	12.69	21	106.99	11.41	18	110.37	14.16	0.512		1.000		0.586
Change		0.56	1.01		-0.17	1.19		1.38	1.69	0.534				
Body Composition														
Fat mass (kg)														
Baseline	42	35.99	14.25	23	37.06	15.32	19	34.70	13.12	0.677				
Mid-Intervention	36	37.51	14.98	18	39.51	15.71	18	35.51	14.37	0.389	0.193	0.396	0.314	0.586
End-Intervention	39	34.72	11.90	21	34.21	10.42	18	35.31	13.72	0.900		0.177		0.556
Change		-0.18	1.30		-0.89	2.01		0.66	1.61	0.112				
Fat free mass (kg)														
Baseline	42	52.18	14.16	23	51.51	16.06	19	52.99	11.84	0.771				
Mid-Intervention	36	53.50	13.43	18	53.48	14.22	18	53.53	13.00	1.000	0.901	0.962	0.311	0.266
End-Intervention	38	52.71	14.10	20	51.55	15.49	18	54.00	12.69	0.613		0.722		0.744
Change		-0.78	1.37		-1.77	2.56		0.31	0.62	0.74				
Fat mass index (kg/m2)														
Baseline	42	13.17	4.85	23	13.66	5.29	19	12.58	4.34	0.553	0.180		0.531	

Mid-Intervention	36	13.37	4.93	18	14.11	5.36	18	12.64	4.49	0.424		0.798		0.756
End-Intervention	39	12.47	3.95	21	12.33	3.68	18	12.64	4.35	0.900		0.214		0.653
Change		-0.28	0.49		-0.67	0.81		0.17	0.49	0.202				
Fat free mass index (kg/m2)														
Baseline	42	19.09	2.81	23	19.13	3.14	19	19.05	2.42	0.934				
Mid-Intervention	36	19.73	6.10	18	18.67	3.63	18	20.79	7.82	0.304	0.640	1.000	0.538	0.794
End-Intervention	39	19.66	5.21	21	20.02	6.76	18	19.24	2.58	0.65		1.000		1.000
Change		0.51	0.78		0.90	1.45		0.06	0.23	0.601				
Skeletal muscle mass (kg)														
Baseline	42	25.47	7.40	23	25.43	8.00	19	25.52	6.82	0.830				
Mid-Intervention	36	25.64	7.69	18	25.66	8.16	18	25.61	7.44	0.888	0.391	0.507	0.269	0.223
End-Intervention	37	25.75	7.46	21	25.04	7.67	16	26.68	7.31	0.514		0.660		0.195
Change		-0.17	0.66		-0.37	1.13		0.09	0.43	0.476				
Visceral Fat (L)														
Baseline	35	3.83	1.92	18	4.44	2.09	17	3.19	1.52	0.017*				
Mid-Intervention	33	4.65	2.75	17	5.15	2.91	16	4.11	2.55	0.127	<0.001*	0.255	<0.001*	0.289
End-Intervention	39	1.65*	1.07	21	1.54	0.62	18	1.78	1.44	0.945		<0.001*		0.001*
Change		-2.22	0.39		-3.05	0.45		-1.40	0.57	0.014*				

LFD, Low-Fat Diet; MedDiet, Mediterranean Diet; SD, Standard Deviation; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, wait-to-hip ratio; NC, neck circumference. All data presented as mean ± SD. * indicates significance ($p < 0.05$).

P^a p-values for comparing differences between participants in each diet group at the respective time point; independent samples t-test for parametric data or Mann-Whitney U test for non-parametric data. P^b p-values for comparing differences within each diet group from baseline to end intervention time points; paired samples t-test for parametric data or Wilcoxon Signed Rank test for non-parametric data. P^c p-values compares differences between baseline, mid-intervention and end-intervention timepoints in the control (LFD) group and P^d compares differences between baseline, mid-intervention and end-intervention timepoints in the intervention (MedDiet) group; one-way repeated measures ANOVA tests were used for parametric data and Friedman tests were used as the non-parametric alternative; post hoc testing was applied using Bonferroni correction (parametric test) or Wilcoxon Signed Rank Test (non-parametric test).

Supplementary Table 4. *Effects of Dietary Intervention on Nutrient and Food Group Intake*

	Total		Control (LFD)		Intervention (MedDiet)		P^a	P^b			
	mean	SD	mean	SD	mean	SD		LFD	P^c	MedDiet	P^d
Macronutrients											
Protein (g)											
Baseline	94.28	34.84	91.20	27.87	98.00	42.29	0.536				
Mid-Intervention	103.16	45.10	107.44	57.23	98.88	29.56	0.577	0.187	0.41	0.86	1.000
End-Intervention	89.27	32.66	83.53	30.08	95.97	35.10	0.241		0.198		1.000
Change	-5.72	34.32	-6.55	25.37	-4.74	43.29					
Carbohydrate (g)											
Baseline	219.22	81.29	203.76	79.94	237.93	81.02	0.178				
Mid-Intervention	200.76	88.82	207.14	98.75	194.37	80.04	0.673	0.184	1.000	0.003*	0.084
End-Intervention	178.08*	73.91	174.24	75.71	182.55	73.66	0.731		0.240		0.05*
Change	-40.71	72.97	-27.22	75.73	-56.44	68.31					
Sugars (g)											
Baseline	87.02	45.97	83.25	43.65	91.59	49.44	0.570				
Mid-Intervention	80.18	66.87	89.37	87.93	70.99	35.98	0.719	0.494	0.446	0.092	0.102
End-Intervention	65.19	27.75	63.17	26.66	67.54	29.57	0.568		0.687		0.777
Change	-23.28	39.37	-22.37	38.16	-24.33	41.83					
Total Fat (g)											
Baseline	83.25	39.54	78.46	33.31	89.04	46.27	0.503				
Mid-Intervention	81.29	40.56	68.61	32.20	93.97	44.82	0.074	0.790	0.187	0.678	0.446
End-Intervention	77.18	41.76	64.09	35.88	92.45	43.87	0.028*		0.943		0.811
Change	-6.88	33.29	-13.82	29.13	1.21	36.75					
Saturated Fat (g)											
Baseline	28.54	14.39	26.92	12.57	30.50	16.47	0.429				
Mid-Intervention	25.01	14.74	23.94	16.46	26.08	13.19	0.669	0.126	1.000	0.136	0.681
End-Intervention	21.98*	11.67	19.81	11.43	24.50	11.75	0.215		0.954		1.000

Change	-6.77	12.65	-6.89	9.30	-6.63	15.99					
Mono-unsaturated Fat (g)											
Baseline	34.00	18.39	31.80	14.82	36.67	22.10	0.658				
Mid-Intervention	35.17	21.69	27.51	13.80	42.84	25.57	0.024*	0.291	0.133	0.846	0.286
End-Intervention	34.60	21.93	26.78	16.10	43.73	24.62	0.009*		0.619		0.777
Change	0.14	17.65	-4.96	14.61	6.10	19.38					
Poly-unsaturated Fat (g)											
Baseline	13.80	8.55	12.92	7.97	14.87	9.30	0.570				
Mid-Intervention	14.39	7.44	11.12	4.79	17.67	8.26	0.010*	0.790	0.679	0.801	0.372
End-Intervention	14.06	8.59	11.33	7.11	17.25	9.25	0.026*		0.372		0.913
Change	0.15	8.81	-1.38	9.60	1.93	7.68					
Cholesterol (mg)											
Baseline	303.29	132.04	282.36	127.77	328.64	136.10	0.161				
Mid-Intervention	288.71	144.62	281.77	112.74	295.64	173.92	0.888	0.589	0.744	0.607	0.286
End-Intervention	274.08	157.62	240.56	106.33	313.19	198.06	0.426		0.758		0.500
Change	-26.72	188.34	-26.05	134.85	-27.50	240.61					
Iron (mg)											
Baseline	11.07	4.77	10.74	4.98	11.47	4.59	0.356				
Mid-Intervention	11.54	4.57	10.31	4.47	12.77	4.46	0.059	0.838	0.983	0.486	0.199
End-Intervention	10.64	4.41	9.65	4.09	11.79	4.60	0.100		0.795		0.327
Change	-0.45	4.06	-1.06	4.67	0.27	3.19					
Zinc (mg)											
Baseline	10.69	4.70	10.50	4.49	10.92	5.05	0.778				
Mid-Intervention	11.73	6.18	12.46	8.21	11.01	3.19	0.491	0.079	0.557	0.708	1.000
End-Intervention	9.55	3.29	8.91	3.55	10.29	2.87	0.195		0.084		1.000
Change	-1.18	4.21	-1.47	3.49	-0.84	5.02					
Potassium (mg)											
Baseline	2944.55	1127.65	3009.61	1195.95	2865.79	1065.99	0.686	0.359		0.978	
Mid-Intervention	3152.29	1881.42	3452.15	2572.16	2852.43	693.75	0.346		0.818		1.000

End-Intervention	2777.31	900.90	2689.61	957.04	2879.63	846.22	0.519		0.455		1.000
Change	-195.23	1158.88	-321.29	1226.97	-48.17	1090.12					
Magnesium (mg)											
Baseline	342.23	145.47	332.61	136.00	353.88	159.17	0.643				
Mid-Intervention	368.91	168.76	365.18	196.77	372.63	141.03	0.897	0.306	1.000	0.958	1.000
End-Intervention	332.68	134.14	308.24	120.94	361.19	146.34	0.224		0.382		1.000
Change	-13.48	132.53	-24.71	157.10	-0.39	99.43					
Sodium (mg)											
Baseline	2409.66	970.86	2410.53	922.43	2408.61	1052.14	0.995				
Mid-Intervention	2372.25	1013.10	2261.41	1015.69	2483.10	1027.36	0.519	0.982	1.000	0.142	1.000
End-Intervention	2145.00	899.82	2202.68	1021.06	2077.70	757.90	0.671		1.000		0.405
Change	-249.64	997.51	-118.42	1028.60	-402.73	966.10					
Low long chain omega-3											
Baseline	0.19	0.34	0.16	0.29	0.23	0.40	0.479				
Mid-Intervention	0.26	0.33	0.16	0.23	0.36	0.38	0.192	0.159	0.722	0.869	0.221
End-Intervention	0.23	0.36	0.13	0.19	0.36	0.46	0.202		0.213		0.965
Change	0.06	0.41	0.01	0.36	0.12	0.47					
Eggs											
Baseline	0.23	0.20	0.19	0.20	0.28	0.19	0.157				
Mid-Intervention	0.17	0.22	0.14	0.24	0.20	0.20	0.400	0.641	1.000	0.153	0.183
End-Intervention	0.19	0.24	0.13	0.14	0.25	0.32	0.148		1.000		1.000
Change	-0.04	0.28	-0.03	0.23	-0.04	0.34					
Meat and meat alternatives											
Baseline	2.88	1.86	2.65	1.57	3.16	2.18	0.471				
Mid-Intervention	3.40	1.56	3.08	1.25	3.72	1.79	0.265	0.204	0.306	0.311	0.215
End-Intervention	2.96	1.63	2.39	1.34	3.61	1.73	0.011*		0.102		0.557
Change	0.03	1.45	-0.20	1.37	0.30	1.52					
Processed Meats											
Baseline	0.14	0.23	0.15	0.26	0.12	0.18	0.919	0.103		0.918	

Mid-Intervention	0.11	0.20	0.11	0.20	0.11	0.21	0.963		0.515		0.638
End-Intervention	0.16	0.23	0.18	0.26	0.14	0.20	0.686		0.114		0.480
Change	0.02	0.28	0.03	0.33	0.02	0.21					
Cheese											
Baseline	0.50	0.67	0.52	0.68	0.46	0.68	0.828				
Mid-Intervention	0.28	0.30	0.27	0.32	0.30	0.28	0.481	0.607	0.352	0.116	0.199
End-Intervention	0.27	0.27	0.26	0.29	0.27	0.26	0.835		0.507		0.210
Change	-0.22	0.65	-0.25	0.70	-0.19	0.60					

Supplementary Table 5. *Partial correlations between inflammatory markers and other inflammatory markers at end intervention, values adjusted for mean weight-loss.*

Correlation Coefficients (adjusted for weight loss)					
	TNF-alpha	IL-6	Adiponectin	Leptin	Resistin
hs-CRP	0.04	0.11	0.11	0.21	.446*
TNF-alpha	-	-0.10	-0.16	0.10	0.10
IL-6	-	-	-0.06	0.13	0.04
Adiponectin	-	-	-	0.30	0.12
Leptin	-	-	-	-	0.19

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). R-values presented; 0.10 - 0.29 weak, 0.30 - 0.49 moderate, 0.50 – 1.00 strong correlations. hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

Supplementary Table 6. *Correlation coefficients for primary inflammatory outcomes and nutrient and dietary food group intake, at the end-intervention timepoint, values adjusted for mean weight-loss*

	Correlation Coefficients (adjusted for weight loss)					
	hs-CRP	TNF-alpha	IL-6	Adiponectin	Leptin	Resistin
Carbohydrate (% of total E)	-0.10	-0.08	0.08	-0.14	-0.03	0.04
Total fat (% of total E)	-0.32	0.13	-0.03	-0.01	0.02	-0.04
Saturated Fat (g)[a]	-0.35	0.02	0.22	-0.16	-0.13	-0.05
Dietary Fibre[a]	-0.31	0.05	-0.20	-0.16	-0.31	-0.10
Cholesterol[b]	0.08	-0.01	0.12	0.05	-0.08	0.20
Alcohol[b]	0.28	-0.20	0.00	-0.10	-0.17	-0.16
Trans Fatty Acids[a]	-0.12	0.13	0.20	-0.10	-0.02	0.01
Vitamin C[b]	0.08	0.28	-0.08	-0.27	-0.14	-0.03
Sodium[a]	-0.20	0.00	-0.14	-0.19	-0.31	-0.09
Calcium[b]	-0.21	0.08	0.15	0.05	-0.20	-0.06
Wholegrains[b]	-0.19	-0.22	-0.08	0.15	-0.12	-0.22
Vegetables[a]	-0.27	0.19	-0.06	-0.25	-0.17	-0.26
Fruit[b]	-0.16	0.00	-0.19	-0.20	-0.29	0.10
Meat and meat alternatives[b]	-0.26	0.17	-0.23	-0.09	-0.31	0.02
Low long chain omega-3[b]	-0.30	0.27	-0.13	0.16	-0.07	-0.08
Legumes[b] (CHO)	-0.26	-0.05	-0.13	-0.17	0.05	0.09
Nuts[b]	-0.22	0.12	-0.09	-0.03	-0.10	0.04
Yoghurt	-0.11	0.01	-0.09	0.09	0.01	0.05
Cheese	-0.10	-0.14	0.09	0.31	0.06	-0.09
Solid fat equivalents[b]	-0.20	-0.05	0.27	-0.15	0.04	0.10
Alcoholic beverages[b]	0.29	-0.19	0.00	-0.09	-0.18	-0.15

Supplementary Material 7. *Point-biserial correlations between body mass index with IL-6 -174 genotypes at end intervention*

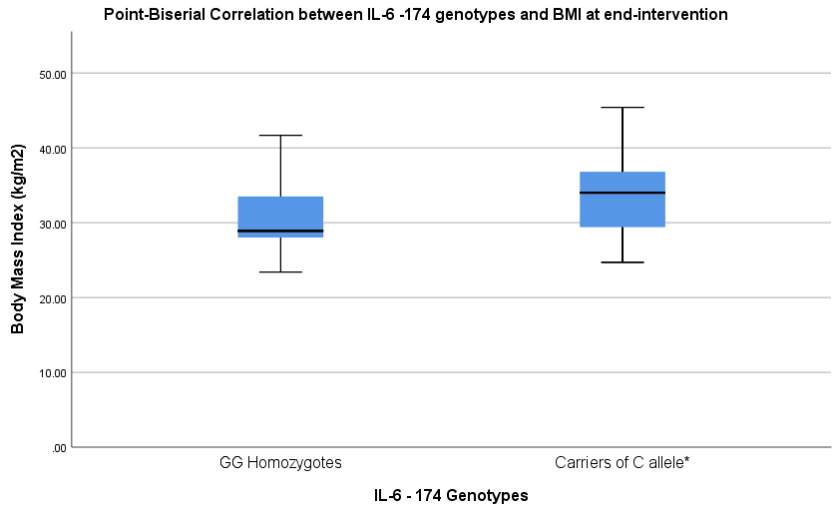
Significant Point-Biserial Correlation's <u>that met the assumptions</u>

Genotype IL-6 -174

Timepoint: end-intervention

Variable: BMI

Point-biserial correlations were run to determine the relationship between variables at each timepoint with IL-6 -174 genotypes. At end intervention, there was a moderate, positive correlation observed between BMI and SNP IL-6 -174, which was statistically significant ($r_{pb} = 0.321$, $n = 39$, $p = 0.047$).



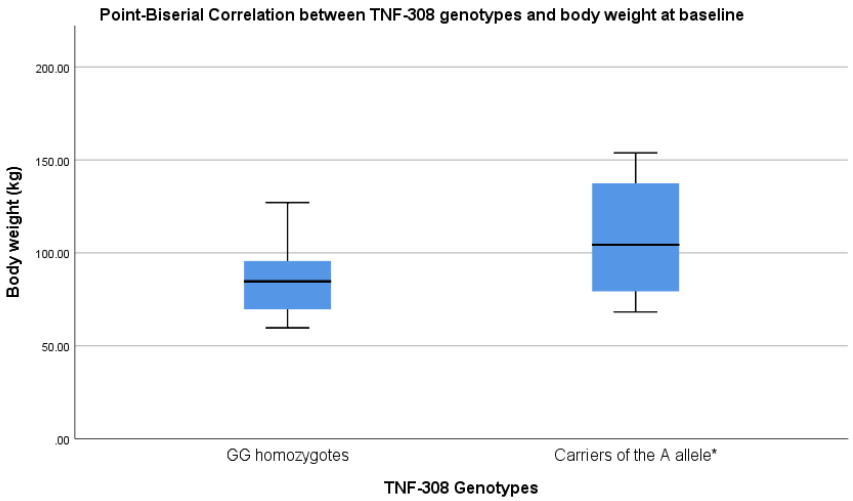
Supplementary Material 8. *Point-biserial correlations between variables at each timepoint with TNF-α -308 genotypes; results are presented as box plots which display the distribution of variables between risk and non-risk genotypes.*

Genotype TNF-α -308

Timepoint: baseline

Variable: weight

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At baseline, there was a moderate, positive correlation observed between body weight (kg) and SNP TNF-α -308, which was statistically significant ($r_{pb} = 0.408$, $n = 42$, $p = 0.007$).

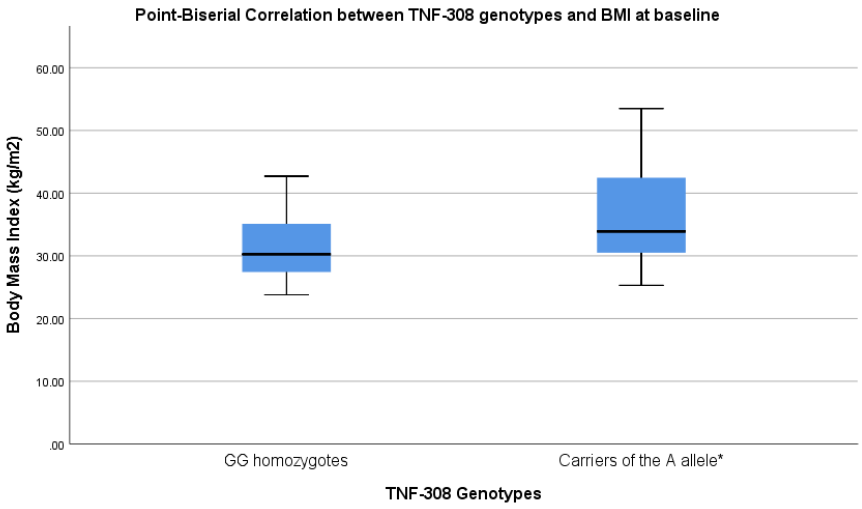


Genotype TNF-α -308

Timepoint: baseline

Variable: BMI

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At baseline, there was a moderate, positive correlation observed between BMI and SNP TNF-α -308, which was statistically significant ($r_{pb} = 0.348$, $n = 42$, $p = 0.024$).

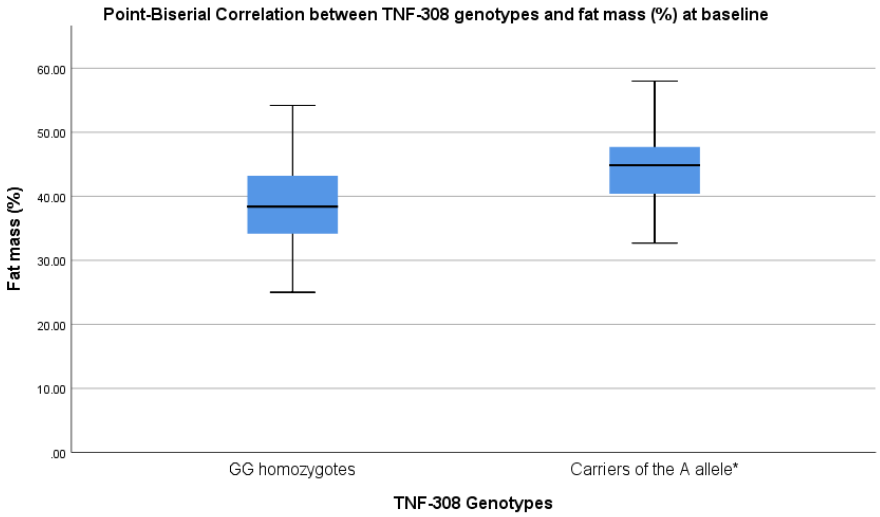


Genotype TNF-α -308

Timepoint: baseline

Variable: fat mass (%)

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At baseline, there was a moderate, positive correlation observed between fat mass (%) and SNP TNF-α -308, which was statistically significant (r_{pb} = *0.356, n = 42, p = 0.021*).

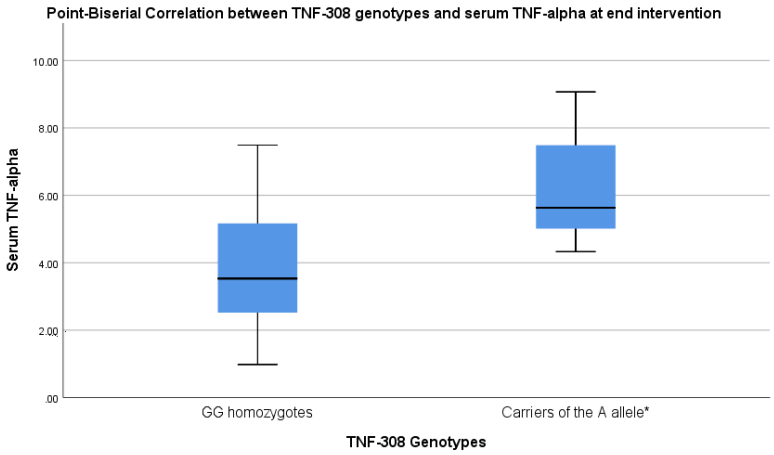


Genotype TNF-α -308

Timepoint: end intervention

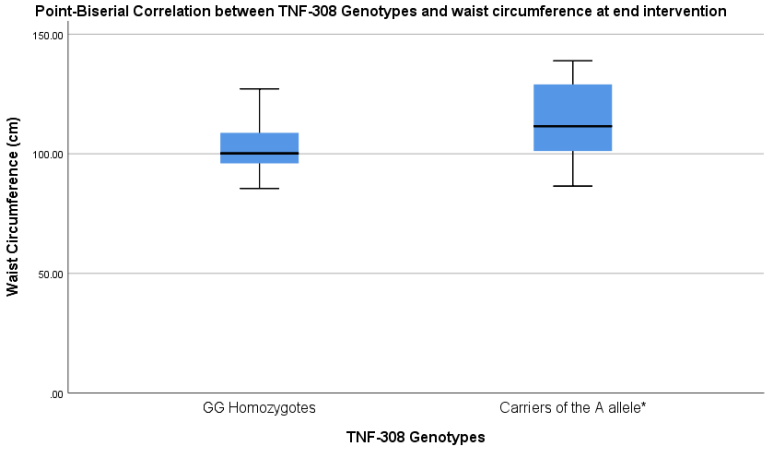
Variable: serum TNF-α

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At end intervention, there was a moderate, positive correlation observed between serum TNF-α and SNP TNF-α -308, which was statistically significant (r_{pb} = *0.493, n = 39, p = 0.001*).



Genotype TNF-α -308

Timepoint: end intervention

Variable: waist circumference

Point-biserial correlations were run to determine the relationship between variables at each timepoint with TNF-α -308 genotypes. At end intervention, there was a moderate, positive correlation observed between waist circumference and SNP TNF-α -308, which was statistically significant ($r_{pb} = 0.321$, $n = 39$, $p = 0.046$).