Original article

# Blunted nutrient-response pathways in adipose tissue following high fat meals in men with metabolic syndrome: A randomized postprandial transcriptomic study

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## ARTICLE INFO

## SUMMARY

*Background:* Excessive adipose tissue is central to disease burden posed by the Metabolic Syndrome (MetS). Whilst much is known of the altered transcriptomic regulation of adipose tissue under fasting conditions, little is known of the responses to high-fat meals.
*Methods:* Nineteen middle-aged males (mean ± SD $52.0 ± 4.6$ years), consumed two isocaloric high-fat, predominantly dairy-based or soy-based, breakfast meals. Abdominal subcutaneous adipose biopsies were collected after overnight fast (0 h) and 4 h following each meal. Global gene expression profiling was performed by microarray (Illumina Human WG-6 v3).
*Results:* In the fasted state, 13 genes were differently expressed between control and MetS adipose tissue ($\geq 1.2$ fold-difference, $p < 0.05$). In response to the meals, the control participants had widespread increases in genes related to cellular nutrient responses ($\geq 1.2$ fold-change, $p < 0.05$; 2444 & 2367 genes; dairy & soy, respectively). There was blunted response in the MetS group ($\geq 1.2$ fold-change, $p < 0.05$; 332 & 336 genes; dairy & soy, respectively).
*Conclusions:* In middle-aged males with MetS, a widespread suppression of the subcutaneous adipose tissue nutrient responsive gene expression suggests an inflexibility in the transcriptomic responsiveness to both high-fat meals.

## 1. Introduction

Adipose tissue (AT) is the major nutrient-storage organ with complex endocrine and immune functions, and is integral to whole-body metabolic health [1,2]. AT expansion, in response to positive energy balance, exerts a central role in the altered nutrient homeostasis, hormonal dysfunction and chronic inflammation that is central to the health burden of non-communicable diseases (reviewed in [3]).

Widespread alterations are evident in the AT transcriptome with metabolic disorders, in comparison to metabolically healthy individuals [4,5]. Yet, these measures have been largely conducted in the overnight fasted state, without providing insight into the transcriptional responsiveness that accompanies a meal ingestion. To date, limited and specific analysis of metabolic and inflammation-related genes has demonstrated transcriptional responsiveness of AT after consumption of a meal [6], but suggests limited meal responses for inflammation-related genes [7]. These

studies further fail to address the global transcriptome, which enables untargeted and systems biology analysis of the transcriptomic flexibility required to transition from a fasting to postprandial metabolic state.

A recent transcriptomic analysis reported that individuals with type 2 diabetes display reduced rhythmicity of AT gene expression, compared with healthy participants [8]. These data complement previous evidence of a metabolic 'inflexibility' that is a key feature of insulin resistance [9,10]. However, major knowledge gaps remain, particularly as to the whether the AT transcriptomic response is modified in individuals at an elevated risk of type 2 diabetes, but prior to the onset of the disease.

Therefore, the aim of this study was to examine the postprandial transcriptome of AT of middle-aged men with and without Metabolic Syndrome (MetS), defined by the International Diabetes Federation (IDF) criteria [11], in response to differing breakfast meals. Global AT gene expression was measured by microarray, before and 4 h following breakfast meals. Two differing high-fat breakfast meals were investigated, composed to be indicative of major patterns of dietary habits, thus included either animal-derived (predominately dairy) or plant-based (predominately soy). It was hypothesized that transcriptomic responsiveness in the adipose from the men with MetS would differ from that of the healthy controls and be characterized by a transcriptomic inflexibility.

## 2. Materials and methods

This trial was registered with the Australian New Zealand Clinical Trial Registry (ACTRN12610000562077) and the methods and primary outcomes have been previously published [12,13]. All participants provided informed, written consent prior to their commencement in the study. All experimental procedures were formally approved by the Deakin University Human Research Ethics Committee (EC-120) in accordance with the Declaration of Helsinki.

### 2.1. Participants and experimental design

Full details of this protocol are published [12]. Briefly twenty-one men aged 40—60 years were recruited and grouped as either MetS according to the 'International Diabetes Federation Criteria for Metabolic Syndrome' [14], or age- and height-matched participants to act as a control group. Participants were non-smokers, and not taking supplements known to interfere with lipid metabolism. Participants were excluded if they showed evidence of acute or chronic inflammatory disease, infectious diseases, cancer, and/or known alcohol consumption (>20 g per day). Participants with type 2 diabetes (as determined by fasting glucose concentration), and people using diabetic medications were also excluded. A randomized, crossover, single meal study was conducted to examine the postprandial effects of two high-fat breakfast meals, either dairy-based or soy-based, that differed in nutrient composition.

Participants fasted overnight then presented to the clinical laboratory at the School of Exercise & Nutrition Sciences, Deakin University, Melbourne, Australia the following morning. Height, weight, and waist circumference were measured with standard procedures. The participants rested for 30 min then an AT sample was collected from the lateral periumbilical region of the subcutaneous abdominal under local anaesthesia (Xylocaine 1% w/v) by percutaneous needle biopsy technique [15] modified to include suction [16]. AT was washed in ice-cold phosphate-buffered saline to eliminate blood and immediately frozen and stored in liquid nitrogen. Participants were fitted with a cannula for collection of fasting (0 h) blood samples, then consumed either a dairy-based or

a soy-based, high-fat breakfast (Table 1) within 15 min. Postprandial AT biopsies were collected at 4 h following the meal ingestion. The biopsies in the current study were taken from a single-entry point (ethical approval was dictated by the Deakin University Human Research Ethics Committee), but each collection point was taken at a 90° angle from the previous biopsy, and at least 4—5 cm from the incision site. Nevertheless, after we observed the results from the initial analysis of the AT microarray, we did note that many inflammatory genes were activated.

Blood was collected and separated for plasma at 60, 120, 180, 240 and 300 min post-meal ingestion, and supplied to Cabrini Pathology (Cabrini Health, Victoria, Australia) for assessment of insulin, glucose, HDL-cholesterol and triglycerides (TG).

Participants returned following a washout period of a minimum of four weeks, and undertook the same procedures for the alternate breakfast.

### 2.2. RNA isolation

AT RNA was extracted using the miRNeasy Mini Kit (Qiagen Inc., Hilden, Germany) following the manufacturers protocol. On-column DNase treatment (Qiagen) was performed. RNA quality and concentration was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA).

### 2.3. Reverse transcription and RT-PCR

Fourteen genes were selected to validate the microarray data (method and data available in Supplementary information file 1). The validation genes were selected based on the initial analysis of the microarray for differentially expressed genes that showed high level of fold change difference from fasting to postprandial: increased in all or most conditions (*CCL2, JUNB, SOD2, PLAU, PLAUR, TNFAIP6, IRF1*); increased in control but not MetS (*NOS3, IL4R*); decreased in all conditions (*PIK3IP1, IRS2*); no change in all groups (*CD68*). Overall, the PCR results consistently demonstrated that the postprandial changes of gene expression levels occurred in the same direction and magnitude as the microarray.

### 2.4. Microarray processing

Illumina Human WG-6 v3 arrays (Illumina, San Diego, CA, USA) which contained probe sets from over 48000 transcripts and variants were used. Microarrays were processed according to the manufacturer's protocols (Illumina, San Diego, CA, USA). The resulting image files were analysed using Illumina Beadstudio software to annotate the genes and generate quantitative expression scores with mean, standard deviation and statistical evaluation

**Table 1**
Nutrient composition of the test meals.

| | Dairy-based meal | Soy-based meal |
|---|---|---|
| Energy (kJ) | 3120 | 3276 |
| Carbohydrate (g) | 37 | 47 |
| Total fat (g) | 54 | 54 |
| - % Energy | 64 | 60 |
| - % Total fat as SFA | 67 | 36 |
| - % Total fat as MUFA | 23 | 39 |
| - % Total fat as PUFA | 5 | 23 |
| Protein (g) | 29 | 29 |
| Calcium (mg) | 672 | 737 |

SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid; Dairy-based meal = cheddar cheese (60 g), butter (20 g) and extra creamy milk (300 mL) with toast (50 g); Soy-based meal = cheddar flavoured soy cheese (100 g), soy beverage (300 mL), a non-dairy spread (20 g) and toast (50 g).

of detection reliability averaged across the 30–50 built in technical replicates for each transcript. The raw and normalized microarray data have been deposited in NCBI Gene Expression Omnibus (GEO series accession number GSE142401).

### 2.4.1. Microarray data pre-processing and quality analysis

The microarray analysis workflow is shown in Fig. 1. An online workflow tool ArrayAnalysis (http://arrayanalysis.org/) was used to perform quality control analysis and process the raw

microarray Illumina data [17]. All arrays passed the criteria of the quality control. A detailed quality control report is available in Supplementary information file 2. Genes that were under-expressed, as determined by a detection *p*-value greater than 0.01 in all samples were filtered out. Data were pre-processed using the functions of the R/Bioconductor packages *lumi* and *limma* [17], background was subtracted and data were quantile normalized. The normalized data were used for all statistical analyses.
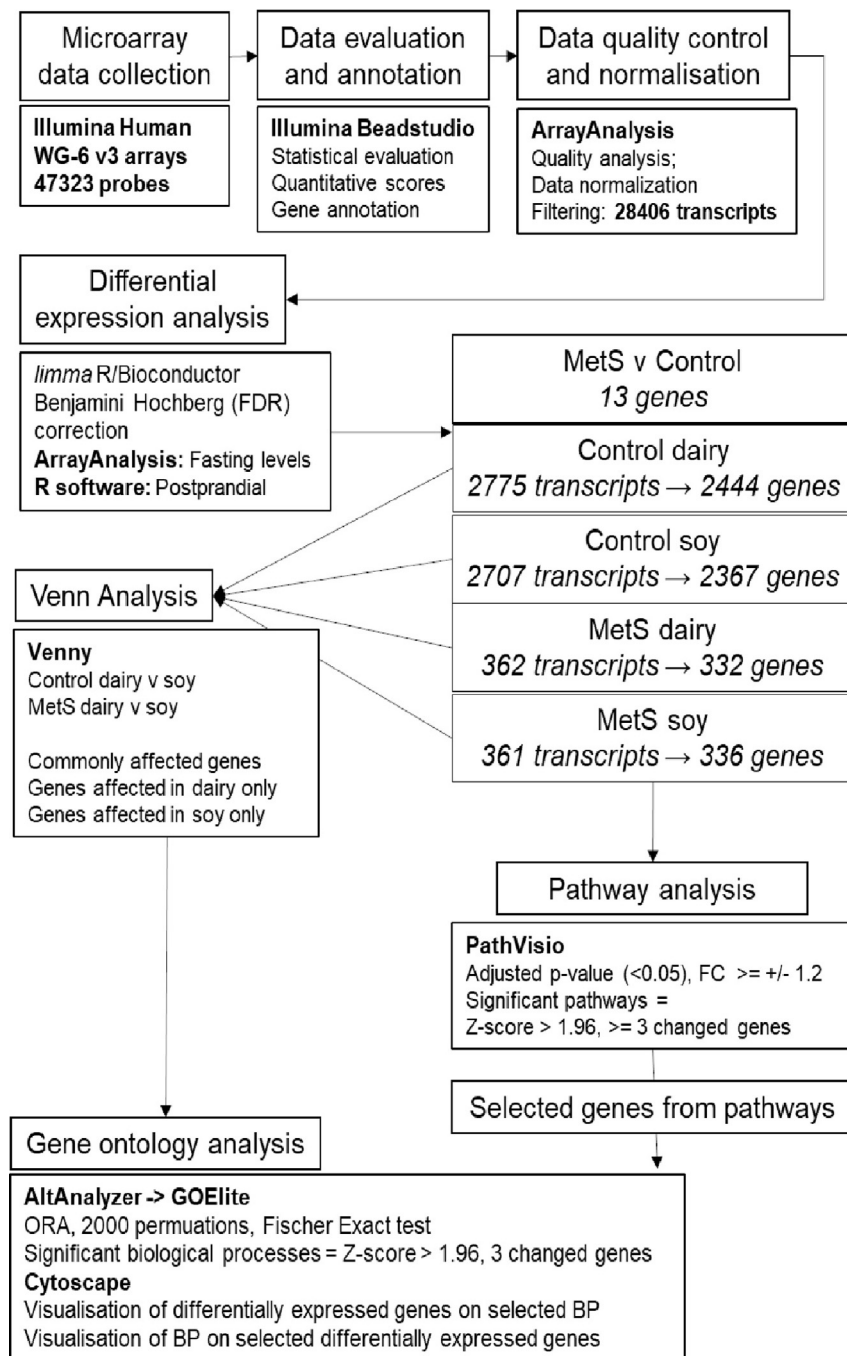


**Fig. 1.** Microarray data analysis workflow. Microarray analysis was performed on adipose tissue samples from men with metabolic syndrome (MetS) and healthy controls before (fasting) and 4 h after either a dairy-based or soy-based high-fat breakfast. After data evaluation, and filtering 28406 transcripts were input to differential expression analysis. MetS v Control = comparison of fasting expression levels between groups; Control dairy = 4 h compared with fasting in control participants after the dairy meal; Control soy = 4 h compared with fasting in control participants after the soy meal; MetS dairy = 4 h compared with fasting in MetS participants after the dairy meal; MetS soy = 4 h compared with fasting in MetS participants after the soy meal.
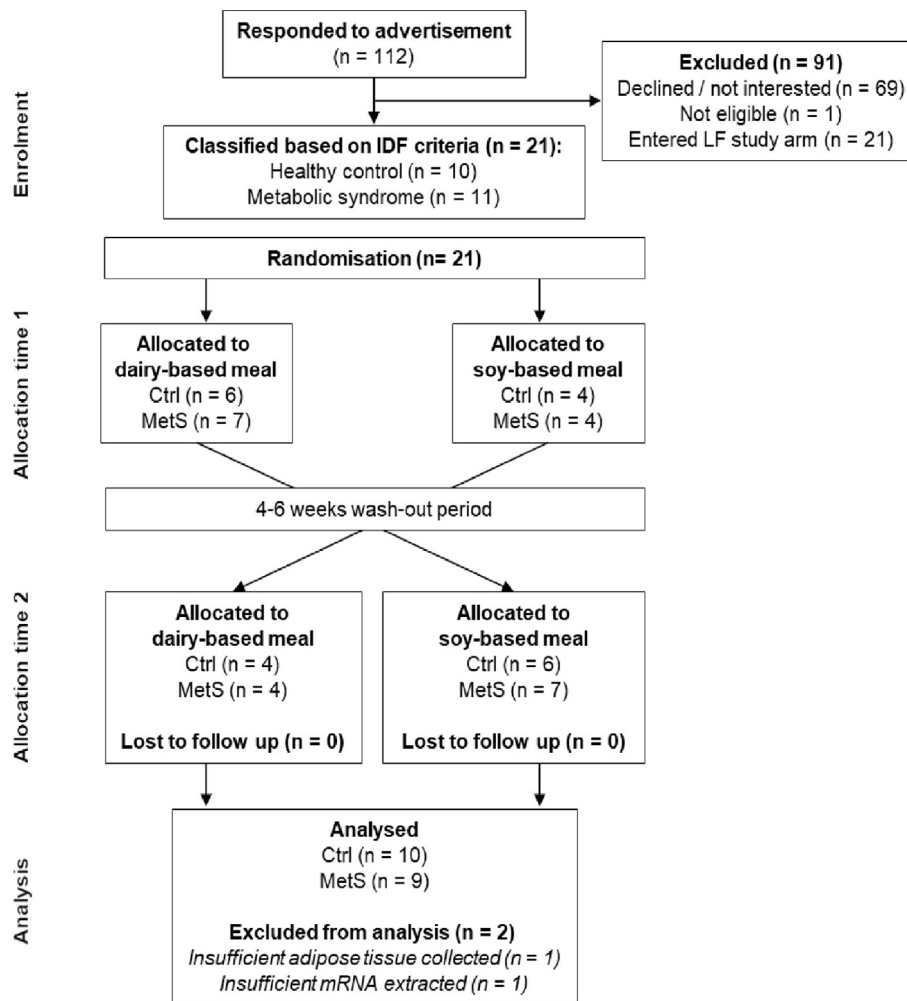
**Fig. 2.** Participant flow through study design. Participants were classified as either healthy or with metabolic syndrome based on the International Diabetes Federation (IDF) criteria for metabolic syndrome [11]. This study was part of a larger trial where participants either entered the high-fat study arm (presented here) or a low-fat (LF) study arm. Ctrl = control men; MetS = men with metabolic syndrome.

### 2.4.2. Differentially expressed genes

Gene expression levels between control participants versus MetS participants at 0 h (fasting) were compared using the statistical module of ArrayAnalysis. This module is based on the *limma* R/Bioconductor package. The analysis included an adjustment for multiple comparisons using the Benjamini Hochberg (FDR) correction.

A paired *t*-test with the *limma* R/Bioconductor package was performed to compare the gene expression levels of AT for each group between 0 h (fasting) and 4 h postprandial.

Gene expression levels were considered differentially expressed if absolute fold-change (FC) $\geq$ 1.2, and adjusted *p*-value < 0.05. Where multiple transcripts for the same gene were differentially expressed, they were reported as a single gene. If a single transcript had significantly changed, the gene was counted.

### 2.4.3. Pathway and gene ontology analysis

The lists of genes that were differentially expressed between fasting and 4 h under each condition were used to find affected processes using an open-source pathway analysis tool, PathVisio v. 3.3.0 [18,19]. For pathway analysis, the criteria selected for over-representation analysis were absolute FC $\geq$ 1.2, and adjusted *p*-value < 0.05. Significantly regulated pathways were determined by a Z-score > 1.96 (2 × SD), and at least 3 changed genes in a pathway.

The numbers of genes that were commonly- or differentially-regulated between controls and participants with MetS in the postprandial state were visualized in Venn diagrams using the freely-available online tool Venny v2.1.0 [20]. Gene ontology analysis was performed to find gene ontology classes per meal. Within the open-source and freely available software suite, AltAnalyzer v2.1.3 [21], the GO-Elite module [22] was used. GO-Elite executes an overrepresentation analysis by calculating a Z-score per GO class using the following filtering parameters; 2000 permutations, significant with a Z-score > 1.96, permuted *p*-value < 0.05 and at least 3 changed genes. To demonstrate connectivity between pathway genes and GO terms, the pruned overrepresentation analysis results were visualized as a network containing links between GO classes and differentially expressed genes that were identified in the pathways to be impacted in control, but not MetS AT with the network analysis tool Cytoscape v3.7.1 [23].

### 2.5. Statistical analysis

As previously reported, sample size was calculated to allow an 80% power to detect a difference in plasma TG concentrations of 0.16 mmol/L between the meals in the control group at 3 h at a significance level of *p* < 0.05 [12]. Random allocation of participants to meal order was performed by MB, using a computer-generated

**Table 2**
Baseline fasting biochemical and anthropometric characteristics at screening.

| | Control $n = 10$ | MetS $n = 9$ | $p$-Value |
|---|---|---|---|
| Age (y) | 51.2 (4.3) | 52.9 (4.9) | 0.437 |
| Height (m) | 1.77 (0.07) | 1.74 (0.04) | 0.273 |
| Weight (kg) | 83.1 (6.0) | 101.6 (18.2) | 0.016 |
| BMI (kg/m²) | 26.6 (2.4) | 33.6 (6.5) | 0.011 |
| FM% | 21.1 (4.3) | 33.4 (6.5) | <0.001 |
| FFM% | 78.9 (4.3) | 68.9 (6.1) | 0.001 |
| Glucose (mmol/L) | 5.38 (0.50) | 5.57 (0.89) | 0.576 |
| HOMA-IR | 1.58 (0.60) | 2.36 (0.92) | 0.041 |
| HDL-C (mmol/L) | 1.29 (0.23) | 1.16 (0.23) | 0.208 |
| TG (mmol/L) | 1.31 (0.28) | 2.14 (0.68) | 0.006 |
| Systolic BP (mm/Hg) | 125 (10) | 137 (17) | 0.067 |
| Diastolic BP (mm/Hg) | 79 (13) | 88 (14) | 0.141 |
| Waist (cm) | 93.6 (7.1) | 111.7 (12.5) | 0.001 |

Values are presented as mean (SD). BMI = body mass index, BP = blood pressure, FFM = fat free mass, FM = fat mass, HOMA-IR = homeostatic model of assessment of insulin resistance, HDL-C = high-density lipoprotein cholesterol, TG = triglyceride. Comparisons were made using independent samples *t*-test. Significance was $p < 0.05$.

random sequence. Participant blinding was not possible, however single blinded analyses were performed for the biological analyses. To determine variability of gene expression levels within the participant groups, the coefficient of variation (CV%) was calculated for expression levels at fasting, and for the change in expression levels from fasting to 4 h postprandial for each gene that was commonly regulated during the postprandial period in both participant groups. Participant baseline biochemical and anthropometric characteristics were assessed for normality using the Shapiro–Wilk test. Independent t-tests were used at baseline to identify differences between participants with MetS and controls for anthropometric, and biochemical measures, and postprandial differences were analysed with repeated measures ANOVA with group (control, MetS) and meal (dairy, soy) as between subject factors and time as a within-subject factor using IBM SPSS Statistics (version 25, SPSS, Chicago, IL).

## 3. Results

### 3.1. Participants' physical and biochemical characteristics

Participant flow through the study design is presented in Fig. 2. Participants in the MetS and control groups were matched for age and height. At screening, participants with MetS had increased weight ($p = 0.016$), fat mass ($p < 0.001$), BMI ($p = 0.011$), waist circumference ($p = 0.001$), and plasma TG ($p = 006$). Fasting levels of glucose, HDL-cholesterol, systolic and diastolic blood pressure were not different (Table 2).

Postprandial biochemical results are presented in Fig. 3. Plasma glucose and insulin results have been previously published [13]. Briefly, postprandial plasma glucose clearance was delayed and insulin levels were increased in participants with MetS compared with control men, with no differences between meals. Postprandial lipids have also been previously published [12]. Postprandial plasma TG concentrations did not differ between groups. Plasma glucose, insulin and TG responses did not differ between meal types for either group. Chylomicron TG content differed between
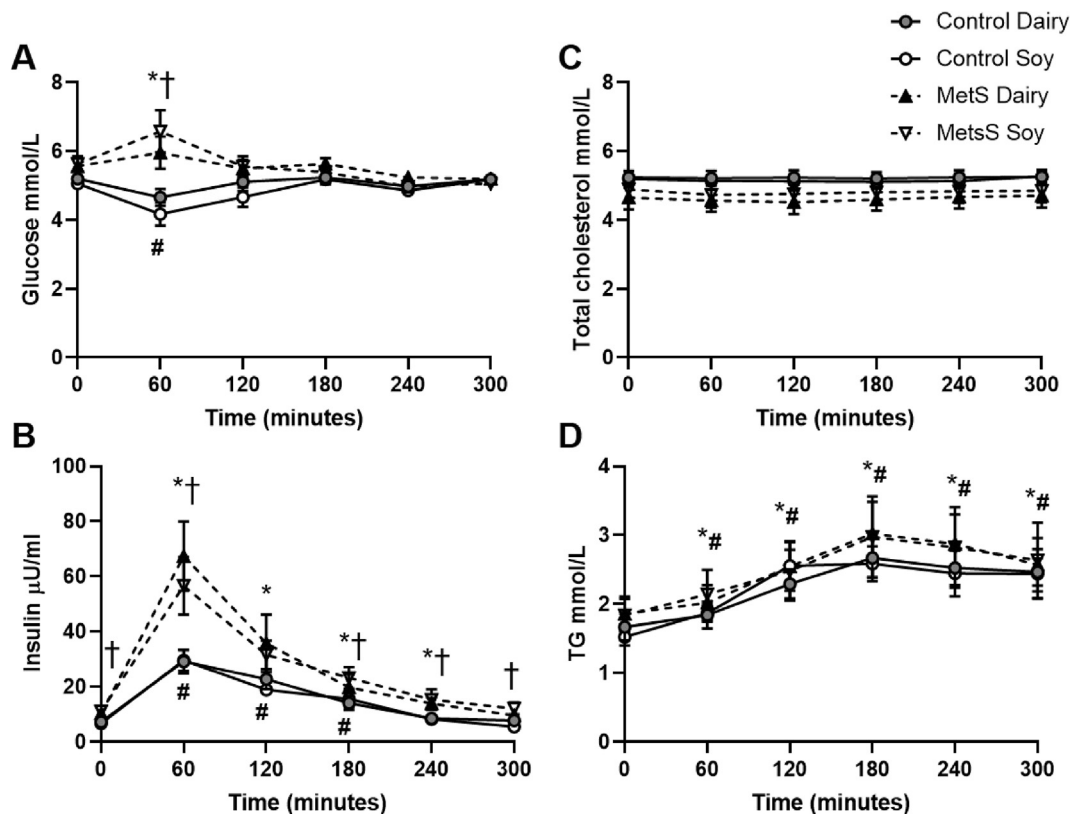


**Fig. 3.** Postprandial responses of plasma glucose, insulin, cholesterol and TG. Glucose (A), insulin (B), were measured in plasma, and cholesterol (C), triglycerides (TG) (D) were measured in serum during the postprandial period after either the dairy or soy meal in participants with metabolic syndrome (MetS) and healthy controls. The data are presented as mean ± SEM, and were analysed for differences using a three-way ANOVA with group (control, MetS) and meal (dairy, soy) as between subject factors and time as a within-subject factor. Significance was set at $p < 0.05$, * = difference from fasting in MetS; # = difference from baseline in controls; † = difference between controls and MetS. Data are adapted from [12,13].

the dairy and soy meals, but were similar between controls and men with MetS [12].

## 3.2. Transcriptomic analysis

### 3.2.1. Adipose tissue gene expression was similar between controls and men with metabolic syndrome in the fasted state

At fasting, only 13 genes were differentially expressed (≥1.2-fold, adj-$p$ < 0.05) in AT between control participants and men with MetS (Supplementary information file 3). Expression levels of nine genes were increased, and four genes were decreased in MetS, compared with control AT. The genes were predominantly associated with proteins that function within the plasma membrane, membranes of vesicles and organelles, or extracellular proteins (Supplementary information file 3). Pathway and gene ontology analyses could not be performed as the number of differentially expressed genes was too low.

### 3.2.2. Postprandial changes in gene expression levels were suppressed in adipose tissue from participants with MetS

After the dairy meal, 2444 genes were differentially expressed (≥1.2-fold, adj-$p$ < 0.05) in control participants compared with fasting. Of these, 1150 genes were increased and 1294 genes were decreased after the meal. In the MetS participants, 332 genes were differentially expressed (268 increased and 64 decreased) after the dairy meal compared with fasting. Postprandial expression levels of 299 genes were commonly regulated after the dairy meal in both control and MetS participants (Fig. 4).

After the soy meal, 2367 genes were differentially expressed (≥1.2-fold, adj-$p$ < 0.05) in control participants compared with fasting. Postprandial expression levels were increased for 1246 genes and decreased for 1121 genes. Whereas, there were 336 genes that were differentially expressed in AT of men with MetS after consumption of the soy meal; 257 genes were increased and 79 genes were decreased. Between the two groups, expression levels of 298 were commonly regulated in AT after the soy meal (Fig. 4).

### 3.2.3. Pathway analysis of postprandial gene expression in adipose tissue

Pathway analysis showed 31 and 32 pathways affected by the dairy meal in AT of control and MetS participants, respectively. Whereas, 62 pathways were overrepresented in control AT, and only 16 pathways affected in MetS AT after the soy meal (Supplementary information file 3).

Based on pathway $Z$-score, heat map representation (Fig. 5), identifies gene pathways that were clustered in and between participant groups. The clustered pathways influenced at 4 h after a meal, regardless of meal composition in AT from the control participants were related to cellular nutrient-response, including; 'TCA cycle nutrient utilization', 'cholesterol biosynthesis', 'PI3K-AKT-mTOR signalling pathway and therapeutic opportunities', 'clear cell renal cell carcinoma' (a pathway related to activation of the mTOR pathway), 'angiogenesis', and 'IL-7 signalling pathway'. These responses were absent in the adipose from the MetS participants.

Several genes were common across the pathways that were changed in control adipose tissue after the meals, but not the MetS, and were associated with cellular growth, metabolism, and nutrient response, and in particular with the mammalian target of rapamycin (mTOR) and ERK signalling pathways (Table 3). Negative regulators of the mTOR pathway (*TSC1*, *TSC2*, *DEPTOR*, *CREBBP*) were decreased in the control adipose tissue, whereas activators, or co-activated pathways were increased (*MAPK1*, *STAT3*, *TGFB3*), which indicates increased activation of the mTOR pathway. Other genes associated with response to external stimuli (*STAT1*, *PIK3R1*, *PIK3R2*)

such as glucose (*HK1*, *HK2*, *SLC2A1*, *PIK3R1*), and amino acids (*SDS*), as well as cell growth and response (*STAT3*, *MAPK1*, *MAPK3*, *TGFB3*, *PDGFRA*, *PDGFRB*) were also impacted in controls but not MetS. In control adipose tissue, *ANGPT1*, which inhibits endothelial permeability was decreased, whereas *NOS3*, responsible for the synthesis of nitric oxide, which increases vascular permeability was increased. This suggests increased blood flow, and nutrient influx in to the tissue.

### 3.2.4. Gene ontology (GO) analysis of the postprandial response of adipose tissue, commonly affected processes and differentially affected processes

Analysis of the 299 commonly regulated genes (Fig. 4) between control and MetS AT after the dairy meal revealed 150 biological processes that were affected (≥3 genes, $Z$-score > 1.96) (Supplementary information file 4). The biological processes with the highest $Z$-score were associated with inflammation and the immune response (Table 4). The postprandial change in AT gene expression levels were similar in direction and magnitude between participant groups.

Gene ontology analysis of the 2145 genes that were altered after the dairy meal in controls but not MetS AT (Fig. 4) identified 132 biological processes that were significantly altered (≥3 genes, $Z$-score > 1.96). Ranked by $Z$-score, the top biological processes were related to cell proliferation, biosynthetic processes, and gene expression regulation (Table 4, Supplementary information file 4). After the dairy meal, only 33 genes were differentially expressed in AT of the MetS group, but not the control participants, GO analyses could not be performed.

After the soy meal 298 genes were commonly regulated in AT of both participant groups (Fig. 4), and were associated with 144 biological processes (≥3 genes, $Z$-score ≥ 1.96). Similar to the
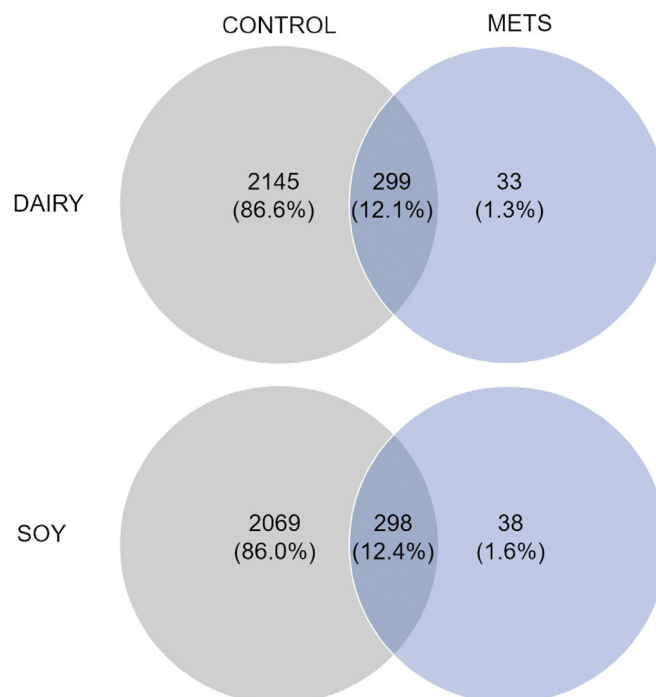


**Fig. 4.** Genes commonly affected during the postprandial period between controls and men with MetS. Venn diagram shows number of genes changed during the postprandial period after either the dairy or soy meal in control participants only (left, grey); both control and MetS participants (centre); and MetS participants only (right, blue).
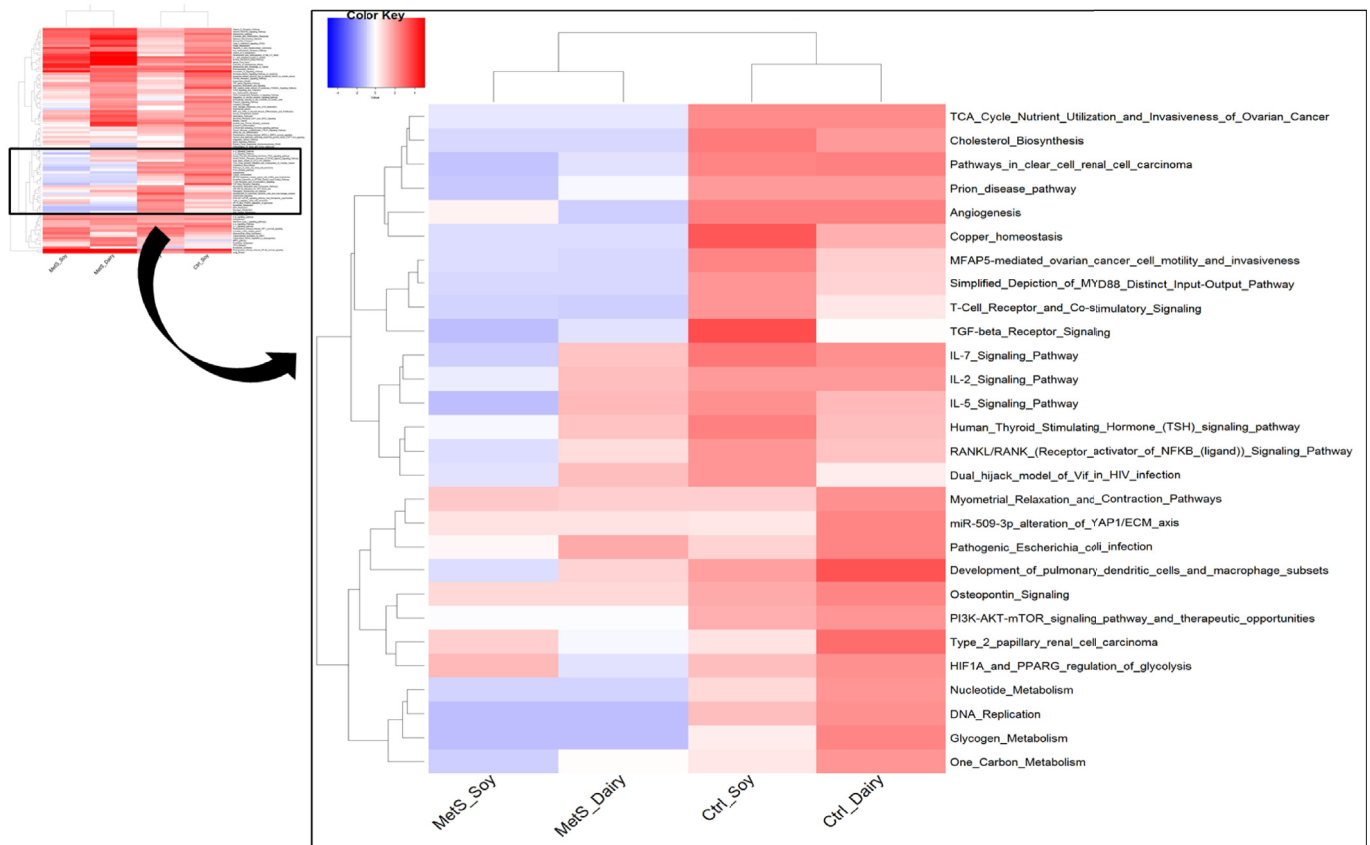
**Fig. 5.** Heatmap of pathways affected in adipose tissue at 4 h after a high-fat meal, based on Z-score. PathVisio v. 3.3.0 [18,19] was used to find affected pathways from differentially expressed (analysis *p*-value (<0.05) and >1.2 fold-change) between fasting and 4 h postprandial in MetS (metabolic syndrome) and Ctrl (control) participants following consumption of either a dairy-based high fat meal or soy-based high fat meal. The heatmap shows pathways that were significantly regulated (Z-score > 1.96, ≥3 positive genes) in at least one group. The cluster shown is pathways that were affected in control adipose tissue, but not MetS adipose tissue in both meal conditions.

commonly-regulated processes after the dairy meal, the common biological processes with the highest *Z*-score after the soy meal were related to inflammation and the immune response (Table 4, Supplementary information file 4). The postprandial change in AT gene expression levels were similar in direction and magnitude between participants with MetS and controls.

There were 2069 genes, and 113 biological processes (≥3 genes, *Z*-score ≥ 1.96) that were differentially expressed in AT after the soy meal in the control group, but not the participants with MetS (Fig. 4). Ranked by *Z*-score, the top biological processes influenced by the soy meal were related to cellular metabolism, and response to external stimuli (Table 4, Supplementary information file 4). As only 38 genes were differentially expressed in AT of the MetS group only after the soy meal GO analyses could not be performed.

Network analysis of genes from the differentially expressed pathways with the affected biological processes for the control participants is shown in Figs. 6 and 7, highlighting gene-pathway connectivity. The biological processes that were the most connected by the highest degree to the genes of interest in control participants aligned with the affected pathways and included; 'response to hormone stimulus', and 'cellular response to organic substance' after the dairy meal; 'regulation of primary metabolic process', and 'regulation of cellular metabolic process' after the soy meal.

### 3.3. Variability of responses

At fasting, there was between 1 and 29% variation between the control participants' expression levels, and 0.6—22% variation in the MetS. The CV of genes demonstrating significant postprandial alteration in both the healthy and MetS groups was similar (299 genes in the dairy meal CVs 26-21952% and 29-9351%; and 298 genes in the soy meal CVs 24-1187% and 23-1107% in controls and MetS, respectively), hence the blunted response observed was a function of gene expression rather that heterogeneity between participants.

## 4. Discussion

In the current study, men with MetS (central obesity plus two or more risk factors [11]) exhibited a markedly suppressed postprandial AT transcriptomic response, at 4 h after high fat breakfasts, compared with the responses in age-matched, healthier men. This suppressed transcriptomic response was consistent and occurred regardless of meal composition. Thus we report an attenuated dynamic transcriptomic response in AT that is consistent with the previously reported phenotypic inflexibility that is evident in individuals who are insulin-resistant, and have heightened metabolic disease risk [24—26].

There were surprising similarities in AT gene expression levels between the control and MetS participants. From the almost 40,000 transcripts measured, only 13 genes were identified to be differentially expressed between participant groups. This was unexpected as given the previous reports that the AT transcriptome is markedly divergent with increased adiposity [4,27—29]. There were smaller differences in fasting risk factors between groups relative to previous studies, which may have contributed to the absence of

**Table 3**
Postprandial changes in expression levels of genes/transcripts that were identified in the pathway analysis to be commonly changed in control adipose tissue, but not MetS after high fat meals.

| Gene symbol | NCBI ID | Illumina ID | Ctrl — Dairy meal | | Ctrl — Soy meal | | MetS — Dairy meal | | MetS — Soy meal | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | FC | Adj p-value | FC | Adj p-value | FC | Adj p-value | FC | Adj p-value |
| STAT3 | 6774 | ILMN_2410986 | 1.66 | 0.002 | 1.58 | 0.005 | 1.37 | 0.105 | 1.24 | 0.171 |
| | 6774 | ILMN_1663618 | 1.53 | 0.006 | 1.51 | 0.006 | 1.33 | 0.122 | 1.21 | 0.199 |
| | 6774 | ILMN_2401978 | 1.49 | 0.010 | 1.53 | 0.009 | 1.26 | 0.443 | 1.10 | 0.689 |
| TGFB3 | 7043 | ILMN_1687652 | 1.50 | 0.048 | 1.98 | 0.003 | 1.39 | 0.231 | 1.27 | 0.369 |
| SLC2A1 | 6513 | ILMN_1659027 | 1.37 | 0.037 | 1.49 | 0.014 | 1.43 | 0.218 | 1.22 | 0.262 |
| HK1 | 3098 | ILMN_2382990 | 1.28 | 0.020 | 1.31 | 0.024 | 1.35 | 0.114 | 1.17 | 0.367 |
| HK2 | 3099 | ILMN_1723486 | 1.55 | 0.002 | 1.58 | 0.001 | 1.35 | 0.115 | 1.15 | 0.378 |
| | 3099 | ILMN_2156172 | 1.29 | 0.063 | 1.31 | 0.015 | 1.09 | 0.754 | 1.16 | 0.379 |
| SDS | 10993 | ILMN_1811114 | 1.46 | 0.039 | 1.75 | 0.012 | 1.30 | 0.566 | 1.07 | 0.880 |
| MAPK1 | 5594 | ILMN_2235283 | 1.44 | 0.008 | 1.25 | 0.014 | 1.22 | 0.187 | 1.15 | 0.320 |
| MAPK3 | 5595 | ILMN_1667260 | −1.27 | 0.045 | −1.25 | 0.016 | −1.13 | 0.549 | −1.17 | 0.172 |
| | 5595 | ILMN_2402341 | −1.28 | 0.047 | −1.24 | 0.025 | −1.01 | 0.969 | −1.12 | 0.436 |
| NOS3 | 4846 | ILMN_1775224 | 2.35 | 0.002 | 1.98 | 0.007 | 1.72 | 0.307 | 1.79 | 0.122 |
| PIK3R2 | 5296 | ILMN_1726565 | −1.29 | 0.013 | −1.30 | 0.031 | −1.27 | 0.322 | −1.20 | 0.230 |
| STAT1 | 6772 | ILMN_1691364 | 1.47 | 0.019 | 1.73 | 0.000 | 1.46 | 0.062 | 1.22 | 0.304 |
| | 6772 | ILMN_1690105 | 1.41 | 0.029 | 1.41 | 0.019 | 1.23 | 0.427 | 1.14 | 0.469 |
| TSC1 | 7248 | ILMN_2246510 | −1.32 | 0.009 | −1.30 | 0.008 | −1.28 | 0.162 | −1.21 | 0.208 |
| | 7248 | ILMN_1797367 | −1.31 | 0.018 | −1.27 | 0.013 | −1.21 | 0.234 | −1.16 | 0.258 |
| TSC2 | 7249 | ILMN_1714216 | −1.21 | 0.032 | −1.23 | 0.037 | 1.20 | 0.332 | −1.20 | 0.176 |
| DEPTOR | 64798 | ILMN_2172755 | −1.64 | 0.005 | −1.37 | 0.045 | −1.30 | 0.300 | −1.15 | 0.412 |
| CREBBP | 1387 | ILMN_1809583 | −1.28 | 0.018 | −1.25 | 0.017 | −1.27 | 0.249 | −1.09 | 0.606 |
| PDGFRA | 5156 | ILMN_2086470 | −1.41 | 0.020 | −1.41 | 0.004 | −1.24 | 0.231 | 1.02 | 0.926 |
| | 5156 | ILMN_1681949 | −1.29 | 0.052 | −1.42 | 0.009 | −1.42 | 0.240 | 1.01 | 0.967 |
| PDGFRB | 5159 | ILMN_1815057 | −1.28 | 0.020 | −1.24 | 0.045 | 1.01 | 0.969 | −1.06 | 0.754 |
| ANGPT1 | 284 | ILMN_1677723 | −1.41 | 0.005 | −1.45 | 0.003 | −1.34 | 0.192 | −1.18 | 0.236 |
| | 284 | ILMN_2086890 | −1.32 | 0.013 | −1.30 | 0.042 | −1.31 | 0.195 | −1.16 | 0.308 |
| CDKN1B | 1027 | ILMN_2196347 | −1.45 | 0.007 | −1.56 | 0.002 | −1.52 | 0.067 | −1.26 | 0.148 |
| | 1027 | ILMN_1722811 | −1.42 | 0.026 | −1.52 | 0.005 | −1.34 | 0.168 | −1.14 | 0.485 |

Adj = Adjusted p-value; Ctrl = Control; FC = Absolute fold change; MetS = Metabolic syndrome. A paired t-test with the limma R/Bioconductor package was performed with Benjamini Hochberg (FDR) correction for multiple comparisons to compare the gene expression levels of AT for each group between 0 h (fasting) and 4 h postprandial after either a high-fat dairy, or high-fat soy meal.

fasting differences in the adipose transcriptome. In examining the 13 genes that were different, no patterns or clustering could be identified. The individual genes that were different with the presence of MetS were associated with proteins that function within the plasma membrane, membranes of vesicles and organelles, or extracellular proteins. Of these, HLA-DMA transcript levels were increased in MetS, consistent with heightened adaptive immune responses in obesity [30,31]. Further, FGF2 gene expression levels were decreased in the MetS individuals. FGF2 is central to adipogenesis [32], and it has previously been shown that adipocytes cultured from participants with MetS exhibit decreased expression levels of FGF2 [33]. Across this population AT fasting gene expression levels did not markedly differ between men with or without MetS suggesting that there were not major differences in the transcriptional regulation of adipocyte function during fasting.

In the present study, participants consumed high-fat (50g; 3000 kJ) breakfast meals, under supervision. These meals resulted in greater postprandial glycaemic and insulinemic responses in the participants with MetS than the controls [13], but no differences in TG responses between groups, although differences were evident between the meals [12]. The meals were matched for total fat and protein content, yet with different nutrient profiles. The animal-derived dairy meal contained more saturated fatty acids, in particular C8:0, C10:0, C12:0 and C14:0. The plant-based soy breakfast contained more mono- and polyunsaturated fatty acids, specifically 18:1(n7) and 18:2(n6) [12]. The dairy meal was also higher in branched-chain amino acids, whereas the soy meal contained increased levels of glycine, alanine, arginine, and aspartic acid [13].

There was global suppression of the postprandial response of AT from participants with MetS (≈ 300 changed genes) compared

with the control participants (≈2500 changed genes) following both meals. Genes related to cellular growth, metabolism, and nutrient response were amongst those that were regulated after the ingestion of the meals only in the control participants. Negative regulators of the mTOR pathway (TSC1, TSC2, DEPTOR, CREBBP) were suppressed whereas activators, or co-activated pathways were increased (MAPK1, STAT3, TGFB3). This suggests gene programming indicative of an increased capacity for mTOR activation. mTOR regulation is central to adipogenesis, postprandial lipid transport and adipose insulin sensitivity [34,35]. Recently the importance of DEPTOR (an mTOR inhibitor) has emerged, with promoter variations of the DEPTOR gene correlating with insulin sensitivity in children and adolescents [36], and elevated AT protein expression levels exhibited in adults with obesity [37]. The present study demonstrated that dynamic postprandial regulation of many genes coding for proteins involved in the complexity of the mTOR signalling cascades occurred in AT following a meal in healthy adult males, which were abolished in men with MetS.

Expression levels of genes related to glucose metabolism were attenuated in men with MetS. These genes included HK1, HK2, and SLC2A1 (formerly GLUT1), which were all increased after both meals in control but not in MetS AT samples. Previously it has been shown that HK2 gene expression levels increased in response to insulin exposure in mouse adipocytes, and diabetic rats display decreased gene expression levels in AT [38]. Interestingly, IL-7 has been shown to stimulate transcription of HK2, resulting in increased glucose uptake via SLC2A1, and cellular glucose retention in T-lymphocytes (immune cells) from mice [39]. The IL-7 signalling pathway was affected in AT from control men after both meals, but not men with MetS.

**Table 4**
Top 15 Biological processes affected (by Z-score) in adipose tissue of control and MetS participants after high-fat meals.

| Commonly affected processes control and MetS | | | Differentially affected processes control only | | |
|---|---|---|---|---|---|
| Ontology-ID | Ontology Name | Z-score | Ontology-ID | Ontology Name | Z-score |
| **Dairy meal** | | | | | |
| GO:0002523 | Leukocyte migration involved in inflammatory response | 15.433 | GO:0016072 | rRNA metabolic process | 5.036 |
| GO:0050729 | Positive regulation of inflammatory response | 10.285 | GO:0031327 | Negative regulation of cellular biosynthetic process | 4.311 |
| GO:0002691 | Regulation of cellular extravasation | 10.170 | GO:0035456 | Response to interferon-beta | 4.243 |
| GO:0032496 | Response to lipopolysaccharide | 9.312 | GO:0033598 | Mammary gland epithelial cell proliferation | 4.243 |
| GO:0010574 | Regulation of VEGF production | 8.441 | GO:0030949 | Positive regulation of VEGF receptor signaling pathway | 4.243 |
| GO:0060326 | Cell chemotaxis | 7.903 | GO:0070344 | Regulation of fat cell proliferation | 4.152 |
| GO:0071496 | Cellular response to external stimulus | 7.745 | GO:0061010 | Gall bladder development | 4.152 |
| GO:0070482 | Response to oxygen levels | 7.529 | GO:0010558 | Negative regulation of macromolecule biosynthetic process | 4.140 |
| GO:0070486 | Leukocyte aggregation | 7.522 | GO:0090083 | Regulation of inclusion body assembly | 4.102 |
| GO:0009612 | Response to mechanical stimulus | 7.521 | GO:0050942 | Positive regulation of pigment cell differentiation | 4.102 |
| GO:0001562 | Response to protozoan | 7.213 | GO:0010629 | Negative regulation of gene expression | 4.042 |
| GO:0002762 | Negative regulation of myeloid leukocyte differentiation | 6.990 | GO:0042401 | Cellular biogenic amine biosynthetic process | 4.028 |
| GO:0045073 | Regulation of chemokine biosynthetic process | 6.985 | GO:0001892 | Embryonic placenta development | 3.994 |
| GO:0034383 | Low-density lipoprotein particle clearance | 6.985 | GO:0048024 | Regulation of nuclear mRNA splicing, via spliceosome | 3.976 |
| GO:0003085 | Negative regulation of systemic arterial blood pressure | 6.985 | GO:0051253 | Negative regulation of RNA metabolic process | 3.814 |
| **Soy meal** | | | | | |
| GO:0070486 | Leukocyte aggregation | 10.257 | GO:0010033 | Response to organic substance | 5.528 |
| GO:0050729 | Positive regulation of inflammatory response | 9.509 | GO:0048519 | Negative regulation of biological process | 5.505 |
| GO:0010574 | Regulation of vascular endothelial growth factor production | 8.527 | GO:0009611 | Response to wounding | 5.257 |
| GO:0030213 | Hyaluronan biosynthetic process | 8.264 | GO:0010939 | Regulation of necrotic cell death | 5.179 |
| GO:0002237 | Response to molecule of bacterial origin | 7.907 | GO:0009607 | Response to biotic stimulus | 5.147 |
| GO:0030593 | Neutrophil chemotaxis | 7.750 | GO:0048518 | Positive regulation of biological process | 5.081 |
| GO:0002523 | Leukocyte migration involved in inflammatory response | 7.597 | GO:0071294 | Cellular response to zinc ion | 5.028 |
| GO:0070555 | Response to interleukin-1 | 7.226 | GO:0031323 | Regulation of cellular metabolic process | 4.694 |
| GO:0071850 | Mitotic cell cycle arrest | 7.055 | GO:0009966 | Regulation of signal transduction | 4.511 |
| GO:0045073 | Regulation of chemokine biosynthetic process | 7.055 | GO:0006928 | Cellular component movement | 4.372 |
| GO:0034383 | Low-density lipoprotein particle clearance | 7.055 | GO:0080090 | Regulation of primary metabolic process | 4.316 |
| GO:0030490 | Maturation of SSU-rRNA | 7.055 | GO:0035284 | Brain segmentation | 4.237 |
| GO:0045933 | Positive regulation of muscle contraction | 6.826 | GO:0008228 | Opsonization | 4.193 |
| GO:0031652 | Positive regulation of heat generation | 6.603 | GO:0009612 | Response to mechanical stimulus | 4.096 |
| GO:0002070 | Epithelial cell maturation | 6.603 | GO:0051704 | Multi-organism process | 3.816 |

AltAnalyzer v2.1.3 [40], the GO-Elite module [41] was used. GO-Elite execute an overrepresentation analysis by calculating a Z-score per GO class using the following filtering parameters; 2000 permutations, significant with a Z-score > 1.96, permuted $p$-value cut-off 0.05 and at least 3 changed genes. Full lists of affected GO classes available in Supplementary File 4.

Despite the similarities in the nutrient-sensing pathways and biological processes that were induced by the two meals, approximately 1000 genes were differentially expressed in AT of control participants after each meal. In both subcutaneous and omental AT of rats it has been previously demonstrated that egg and dairy proteins that differ in amino acid composition differentially impact postprandial expression levels of phosphorylated hormone-sensitive lipase, a protein involved in fat metabolism [40]. Furthermore, components of dairy such as whey and casein, and varied compositions of milk fat also have differentially impacted postprandial levels of genes associated with fat metabolism in AT of human adults [41]. As the meals differed in nutrient profiles, the differences observed in the present study are likely due to the varied response of AT to different stimuli. Further studies are needed to determine whether these differences have an impact on health outcomes.

In this study, genes that were commonly affected in controls and men with MetS during the postprandial period were related to inflammatory and immune biological processes. Postprandial inflammatory gene expression levels have previously been reported to be increased in AT of men with MetS regardless of fatty acid composition of the meal [42], and also in men with obesity, however with reduced expression levels observed after consumption of canola oil compared with olive oil [43]. These commonly affected

processes may have been a normal response to meals, however previous work clearly showed that it is due to the tissue collection method itself [6]. Even in the absence of a meal, repeated AT biopsies have shown to increase expression levels of inflammatory genes [6]. Therefore, the commonly affected genes are potentially due to the procedural effect, whereas differentially regulated genes are more likely to be due to the impact of the meal. Any small meal-induced changes in these gene expression levels, are likely to be masked by the large changes induced by the biopsy procedure.

There are several notable limitations and complexities of the current study. The analysis was limited to analysis of global gene expression, thus, it is not possible to draw definitive conclusions as to the dynamic regulation of both the AT proteome and post-translational regulation of signalling pathways. This study also only reported on changes in gene expression at a single post-prandial time point (4 h), which limits the insight into the dynamics of the response, and the scope of the conclusions that can be drawn. However, the subcutaneous AT biopsy protocol is invasive, and participants experience localized discomfort, so it is not possible using the current technique to more frequently sample without adding to this participant burden.

In conclusion, men with MetS, predominantly characterized by increased levels of adiposity, demonstrated a blunted postprandial
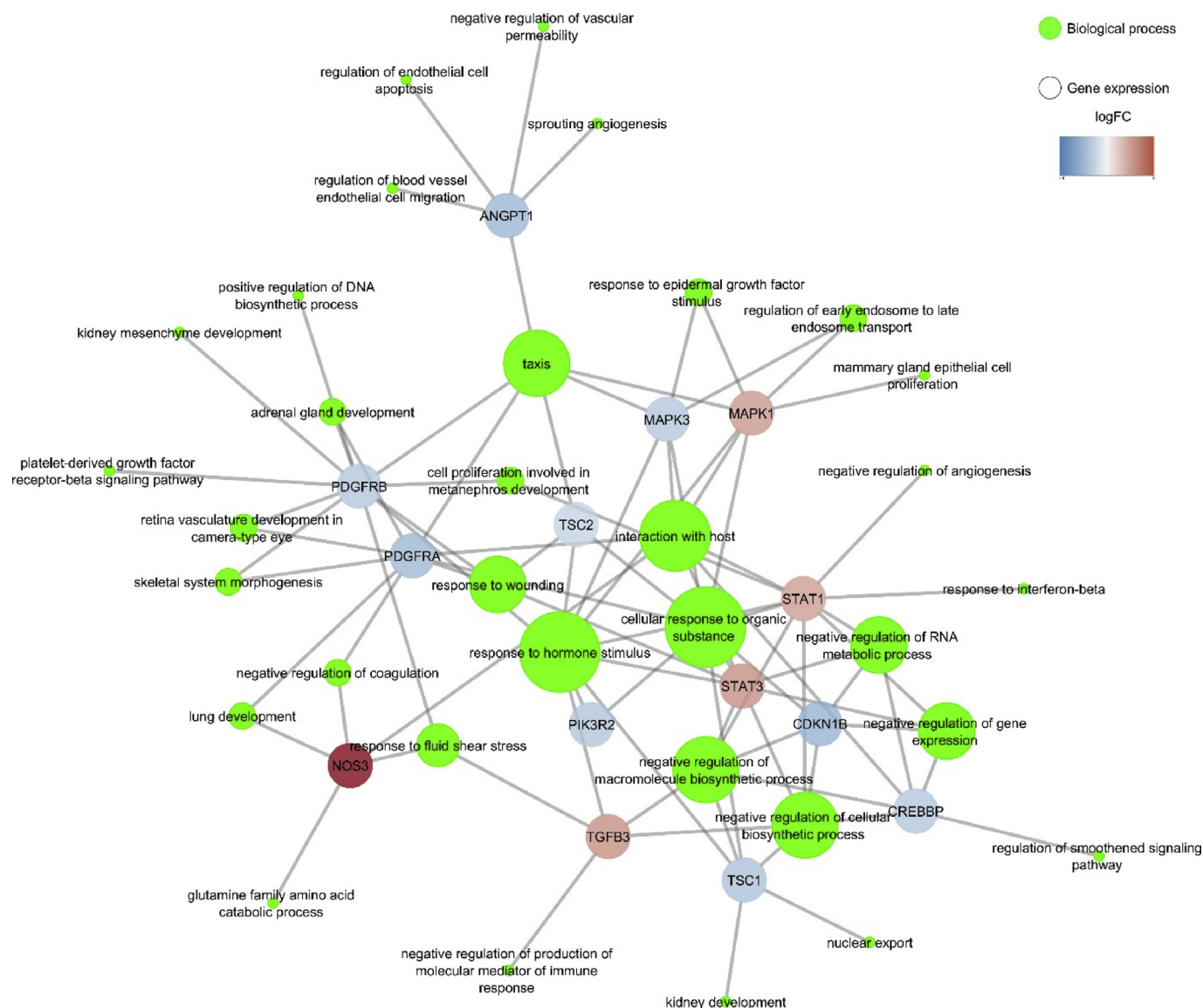
**Fig. 6.** Network of selected genes with affected biological processes (dairy). Selected genes from pathway analysis in control adipose tissue after the dairy meal were visualized as a network containing links between GO classes and differentially expressed genes with Cytoscape v3.7.1 [23]. (AltAnalyzer v2.1.3 [21], the GO-Elite module [22] was used; 2000 permutations, significant with a z-score > 1.96, permuted p-value 0.05 and at least 3 changed genes); Green = affected biological processes Red = gene expression increased after the meal; Blue = gene expression decreased after the meal; Node size depicts degree of connectivity, larger node connects with higher number of selected genes.

transcriptome response in AT at 4 h compared with healthy controls. Phenotypic flexibility is generally demonstrated via markers in circulation [9,10,44], however this study uniquely demonstrates an inability of AT from people with MetS to respond to postprandial challenges at a tissue level, regardless of meal composition. This tissue-level phenotypic inflexibility may have been a result of insulin resistance in the men with MetS, however the changes may precede observable disruptions in postprandial lipid clearance. Further exploration in to the role of mTOR and related signalling pathways in AT may provide insight into mechanisms that could be useful therapeutic targets to improve metabolic health in people with MetS.

## Author statement

**ALD:** Formal analysis, Methodology (microarray analysis), Investigation, Writing — Original Draft; **SLC:** Formal analysis, Methodology (microarray analysis), Visualization, Writing —

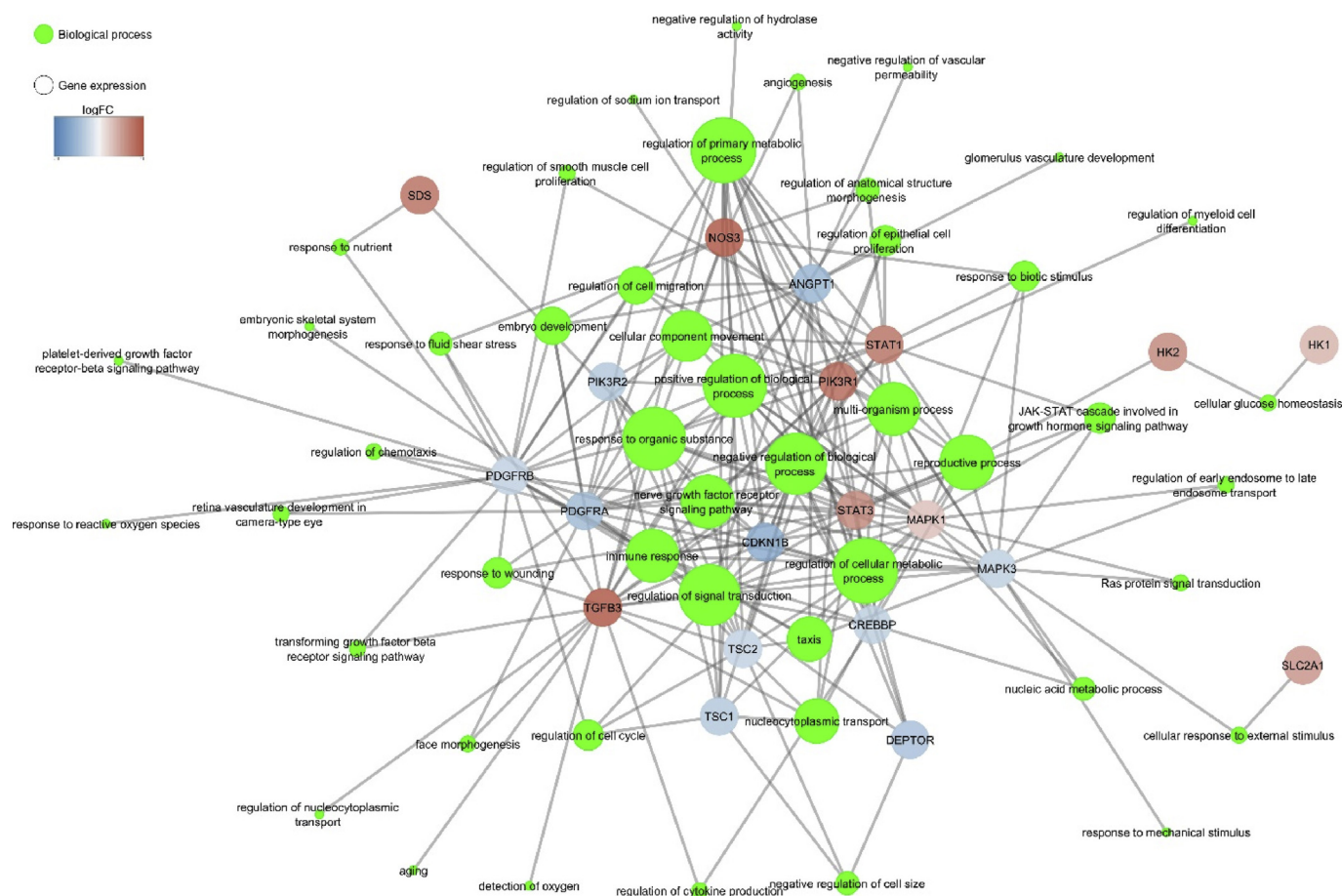**Fig. 7.** Networks of selected genes with affected biological processes (soy). Selected genes from pathway analysis in control adipose tissue after the soy meal were visualized as a network containing links between GO classes and differentially expressed genes with Cytoscape v3.7.1 [23]. (AltAnalyzer v2.1.3 [21], the GO-Elite module [22] was used; 2000 permutations, significant with a $Z$-score > 1.96, permuted $p$-value 0.05 and at least 3 changed genes); Green = affected biological processes Red = gene expression increased after the meal; Blue = gene expression decreased after the meal; Node size depicts degree of connectivity, larger node connects with higher number of selected genes.

Review and Editing; **CTE:** Formal analysis, Methodology (microarray analysis), Writing — Review and Editing; **CM:** Formal analysis, Writing — Review and Editing; **AJS:** Conceptualization, Funding Acquisition; Supervision, Writing — Review and Editing; **AEL:** Investigation, Writing — Review and Editing; **PG:** Investigation, Writing — Review and Editing; **MPB:** Project administration, Investigation, Writing — Review and Editing; **DCS:** Conceptualization, Methodology (trial); Funding Acquisition; Supervision, Writing — Review and Editing.

## Conflict of interest

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.08.024.

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