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APPENDIX 1 ETHICS

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APPENDIX 1 ETHICS

1. DECLARATION FOR EXTERNAL SUPERVISOR/INVESTIGATORS

DECLARATION FOR EXTERNAL INVESTIGATOR

INVESTIGATOR DETAILS AND ROLE IN THE PROJECT

Name: Dr. Katsardis Charalambos

Professional Status: Paediatric Respiratory Physician

Address: Pafou 5, Maroussi, Athens, Greece

Home telephone: 0030-210-9322946

Mob Tel No. : 0030-6946631371

E-mail: katsardis@yahoo.gr

Role: Co-investigator

Project Title: The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in asthmatic children

Declaration For External Investigator

I, C. KATSARDIS declare that:

- ☐ I have no financial or non-financial interests, which may inappropriately influence me in the conduct of this research study.
- ☐ I am suitably qualified and experienced to perform all clinical evaluations regarding Asthma in children.
- ☐ I agree to timeously report to the Ethics Committee serious adverse events that may occur in the course of the investigation
- ☐ I accept to support the research project with my advice based on well- documented medical knowledge.
- ☐ I agree to allow Mrs Papamichael to be present during medical examinations of children in order to distribute questionnaires to children's parents and register the appropriate information/data (including demographics) and to provide her all the available measurements (anthropometric, spirometry, biochemical) conducted during the study.

APPENDIX 1 ETHICS

APPENDIX 1 ETHICS



La Trobe University
School of Allied Health
Dept. of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia
Tel: 0061394793640

DECLARATION FOR EXTERNAL INVESTIGATOR

INVESTIGATOR DETAILS AND ROLE IN THE PROJECT

Name: Dr. Dimitris Tsoukalas
Professional Status: Medical Doctor, Expert in Nutritional Medicine
Address: Koumbari 5, Kolonaki, Athens, Greece
Home telephone: 0030-210- 3611054
E-mail: iatreio@drtsoukalas.com

Role: Co-investigator

Project Title: The prophylactic potential of a Mediterranean dietary pattern enriched with fatty fish in asthmatic children

Declaration For External Investigator

I, DIMITRIS TSOUKALAS declare that:

- ☐ I have no financial or non-financial interests, which may inappropriately influence me in the conduct of this research study.
- ☐ I am suitably qualified and experienced to perform all clinical evaluations regarding asthma in children.
- ☐ I agree to timeously report to the Ethics Committee serious adverse events that may occur in the course of the investigation
- ☐ I accept to support the research project with my advice based on well- documented medical knowledge.
- ☐ I agree to provide Maria Papamichael all the biochemical measurements conducted during the study.

Signature:..... Date: 10/10/2016..... (Athens)

APPENDIX 1 ETHICS

DECLARATION FOR EXTERNAL SUPERVISOR

INVESTIGATOR DETAILS AND ROLE IN THE PROJECT

Name: Professor Michael Koutsilieris

Professional Status: Professor of Experimental Physiology,

Address: National Kapodistrian University of Athens, Dept of Medicine, Greece

Tel: 210-7462597

Mob Tel No.: 0030-6944835200

E-mail: mkoutsil@med.uoa.gr

Role: Supervisor

Project Title: The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in asthmatic children.

Declaration for External Supervisor

I,Professor....Michael Koutsilieris.....declare that:

I accept to support the research project with my advice based on medical knowledge. This will be held under the collaboration of the University of Athens with Latrobe University, Melbourne.

Signature:



Date: 12/4/2016 (Athens)

APPENDIX 1 ETHICS

2. LA TROBE UNIVERSITY ETHICS APPROVAL

Application HEC16-035 (Finalised - Approved)

ResearchMasterEthics@latrobe.edu.au

7/7/2016

ResearchMasterEthics@latrobe.edu.au;
C.Itsiopoulos@latrobe.edu.au;
18782948@students.latrobe.edu.au

LA TROBE ETHICS

Dear Catherine Itsiopoulos,

The following project has been assessed as complying with the National Statement on Ethical Conduct in Human Research. I am pleased to advise that your project has been granted ethics approval and you may commence the study.

Application ID: HEC16-035

Application Status/Committee: Finalised - Approved

Project Title: The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in asthmatic children

Chief Investigator: Catherine Itsiopoulos

Other Investigators: Charis Katsardis, Maria Papamichael

Date of Approval: 08/07/2016

Date of Ethics Approval Expiry: 31/12/2018

APPENDIX 1 ETHICS

3. REGISTRATION APPROVAL WITH THE AUSTRALIAN & NEW ZEALAND CLINICAL TRIALS REGISTRY

Your ACTRN (registration number): ACTRN12616000492459p

info@actr.org.au

To: sassipap@hotmail.com

Dear Maria Papamichael,

Re: The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in improving respiratory function in asthmatic children.

Thank you for submitting the above trial for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR).

Your trial has now been successfully registered and allocated the ACTRN:
ACTRN12616000492459p

Web address of your trial: <http://www.ANZCTR.org.au/ACTRN12616000492459p.aspx>

Date submitted: 5/04/2016 3:09:58 PM

Date registered: 14/04/2016 11:23:44 AM

Registered by: Maria Papamichael

If you have already obtained Ethics approval for your trial, could you please send the ANZCTR a copy of at least one Ethics Committee approval letter? A copy of the letter can be sent to info@actr.org.au (by email) OR (61 2) 9565 1863, attention to ANZCTR (by fax).

Please be reminded that the quality and accuracy of the trial information submitted for registration is the responsibility of the trial's Primary Sponsor or their representative (the Registrant). The ANZCTR allows you to update trial data, but please note that the original data lodged at the time of trial registration and the tracked history of any changes made will remain publicly available.

The ANZCTR is recognised as an ICMJE acceptable registry (<http://www.icmje.org/faq.pdf>) and a Primary Registry in the WHO registry network (<http://www.who.int/ictrp/network/primary/en/index.html>).

If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

Kind regards,
ANZCTR Staff

T: +61 2 9562 5333; F: +61 2 9565 1863 E: info@actr.org.au

W: www.ANZCTR.org.au

APPENDIX 2A STUDY MATERIALS

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1a) Letter to supermarkets inviting them to participate in this project



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

Athens, 29.7.16

To whom it may concern,

Funding for research project titled “The prophylactic potential of a Mediterranean Diet enriched with fatty fish in asthmatic children.”

Dear Sir/Madame,

In Greece, asthma has become one of the most common chronic diseases in children. Research has documented that adherence to a Mediterranean dietary pattern and consumption of fatty fish seems to protect and reduce asthma in children [Anthrakopoulos, 2007; Arvaniti, 2011; Chatzi, 2007].

A clinical trial focusing on the effect of fatty fish intake on pulmonary function of Greek asthmatic children 5-12 years, will be undertaken by Maria Papamichael, Dietician, PhD Candidate La Trobe University, Melbourne in association with a paediatric asthma specialist.

Study Outline

The intervention group will consist of 32 children who will be instructed to consume two fatty fish meals weekly (150g cooked per serve) based on the Hellenic Nutritional Guidelines (Prolepsis, 2014), over a 6 month period including pulmonary function testing.

Estimation of total amount of fatty fish required for implementation of the project

The estimated amount of fatty fish required to be consumed by each child on a monthly basis is 3.5 Kilos, that is 21 Kilos for 6-months. Hence, approximately 672 Kilos of fatty fish will be required for a total of 32 participants over a 6 month period. Fatty fish includes the following types of fish: sardines, salmon, trout, gilthead sea bream, mackerel, anchovies and chubb mackerel.

Timeline for study implementation:

It is anticipated that the study will commence on 1/10/2016 until 30/5/2017. We are inviting your company to contribute to this study by providing the participants with the total amount of fatty fish required for the 6-month period. If your company decides to support this project, we would be most happy to discuss details concerning distribution of the fish to all families.

Over the years, your company has shown social responsibility and sensitivity especially when children are concerned, and has been involved in a number of projects for scientific purposes. With this letter we invite your company to become a sponsor for this study and benefit from the following:

- ✓ Company logo will be advertised during: presentations, publications of the study design and results in Greek and international conferences (as communications and posters)
- ✓ Company logo will be printed on all questionnaires completed by parents of participating children
- ✓ Your company will be advertised during television and radio station interviews nationwide, on on-line media and electronic newspapers.

We hope to hear from you soon.

For any queries/clarification, please do not hesitate to contact one of the members of the research team.

Research Group

Pediatric Pneumologist: Dr. Ch. Katsardis, 210-9322946, katsardis@yahoo.gr

Chief Supervisor: Assoc. Prof. C. Itsiopoulos, 0061394793640, c.itsiopoulos@latrobe.edu.au

Researcher: Maria Papamichael, Dietitian (RD), 6947-073672, sassipap@hotmail.com

1b) Letter to supermarkets inviting them to participate in this project (Greek version)

School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne. 3086. Australia

Αθήνα 29.7.16

Προς τον ενδιαφερόμενο χορηγό

Οικονομική Υποστήριξη της ερευνητικής μελέτης με τίτλο «Η θεραπευτική δράση της Μεσογειακής διατροφής σε παιδιά που πάσχουν από άσθμα»

Αγαπητέ κύριε/κυρία,

Στην Ελλάδα, το άσθμα αποτελεί μία από τις πιο συχνές χρόνιες ασθένειες σε παιδιά, αλλά σύμφωνα με μελέτες, η μεσογειακού τύπου διατροφή και η κατανάλωση λιπαρών ψαριών, φαίνεται να δρα προφυλακτικά με μείωση των συμπτωμάτων [Anthracopoulos, 2007; Arvaniti, 2011; Chatzi, 2007].

Μια ερευνητική κλινική μελέτη για την θετική επίδραση της κατανάλωσης λιπαρών ψαριών στην πνευμονική λειτουργία ασθματικών ελληνόπουλων ηλικίας 5-12 ετών, θα πραγματοποιηθεί από τη Μαρία Παπαμιχαήλ, Διαιτολόγο, Υποψήφια διδάκτωρ του Πανεπιστημίου La Trobe της Μελβούρνης, σε συνεργασία με ιατρούς εξειδικευμένους στην αντιμετώπιση του παιδικού άσθματος.

Σύντομη περιγραφή του έργου

32 παιδιά που θα ανήκουν στην ομάδα παρέμβασης θα κληθούν να καταναλώνουν 2 φορές την εβδομάδα τη συνιστώμενη ποσότητα λιπαρού ψαριού [Εθνικός Διατροφικός Οδηγός (Prolepsis), 2014], για μια περίοδο 6 μηνών, συνοδευόμενα από αξιολόγηση της πνευμονικής τους λειτουργίας.

Υπολογιζόμενη συνολική ποσότητα λιπαρών ψαριών για την υλοποίηση της ερευνητικής μελέτης

Η ποσότητα λιπαρών ψαριών που αναμένεται να καταναλώνει κάθε παιδί μηνιαίως είναι 3,5 κιλά και επομένως 21 κιλά το εξάμηνο. Επομένως για το σύνολο των συμμετεχόντων παιδιών (32) καθ'ολη τη διάρκεια των 6 μηνών η απαιτούμενη ποσότητα είναι 672 κιλά περίπου λιπαρών ψαριών. Ας σημειωθεί ότι στα λιπαρά ψάρια συγκαταλέγονται η σαρδέλα, ο σολομός, ο γαύρος, η πέστροφα, η τσιπούρα, ο κολιός, και το σκουμπρί.

Χρονική περίοδος υλοποίησης

Η μελέτη αναμένεται να διαρκέσει από 1/10/2016 έως 30/5/2017.

Για το χρονικό αυτό διάστημα των 6 μηνών η εταιρεία θα θέλαμε να παρέχει το σύνολο της ποσότητας των λιπαρών ψαριών στις οικογένειες των συμμετεχόντων παιδιών. Ο τρόπος χορήγησης των ψαριών θα συζητηθεί στην περίπτωση που η εταιρεία σας αποφασίσει να ενισχύσει το έργο μας.

Με τα χρόνια η εταιρεία σας έχει αποδείξει την κοινωνική ευθύνη που την διέπει και ευαισθητοποιείται ιδιαίτερα όταν πρόκειται για παιδιά, με την συνεισφορά της σε δραστηριότητες για επιστημονικούς σκοπούς. Με την παρούσα επιστολή σας προσκαλούμε να γίνετε χορηγός της μελέτης απολαμβάνοντας τα εξής προνόμια:

- ✓ Παρουσίαση του εταιρικού logo σε όλες τις δημοσιεύσεις του σχεδιασμού και των αποτελεσμάτων της μελέτης σε ελληνικά και διεθνή συνέδρια (ανακοινώσεις και αναρτήσεις)
- ✓ Εκτύπωση του εταιρικού logo στα ερωτηματολόγια που θα συμπληρώνουν οι γονείς των συμμετεχόντων παιδιών
- ✓ Διαφήμιση της εταιρείας σας σε όλες τις δημοσιεύσεις σε τηλεοπτικούς και ραδιοφωνικούς σταθμούς πανελλαδικής εμβέλειας, on line μέσα ενημέρωσης και ηλεκτρονικές εφημερίδες

Ελπίζουμε να έχουμε σύντομα νέα σας.

Για οποιαδήποτε απορία/ διευκρίνιση μη διστάσετε να επικοινωνήσετε με οποιοδήποτε μέλος της ερευνητικής ομάδας.

Η ερευνητική ομάδα

Ο παιδοπνευμολόγος: Δρ. Χ. Κατσαρδής, 210-9322946, katsardis@yahoo.gr

Η επιβλέπουσα Καθηγήτρια: Δρ. Κ. Ιτσιόπουλος, 0061394793640, c.itsiopoulos@latrobe.edu.au

Η ερευνήτρια: Μαρία Παπαμιχαήλ Διαιτολόγος, 6947-073672, sassipap@hotmail.com

2a) Participant Information Sheet



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

Dr. Ch. Katsardis
Pafou 1, Marousi, Athens, 15125

INFORMATION SHEET

A research study on “The prophylactic potential of a Mediterranean diet enriched with fatty fish on Greek asthmatic children” will be undertaken by Maria Papamichael, Dietitian, PhD Candidate La Trobe University, Melbourne, under the supervision of Assoc. Prof. C. Itsiopoulos and in association with Paediatric Pneumologist, Dr.Ch. Katsardis.

Globally, asthma has become one of the most frequent chronic diseases in children, caused by genetic and environmental factors. Recent studies have suggested that adherence to a Mediterranean-type diet and consumption of fish may prevent or reduce asthma symptoms in children.

Therefore, the aim of this proposed study is to examine if the consumption of fatty fish improves pulmonary function and reduces episodes and severity of asthma in children. The findings of this study will help to design nutritional guidelines for the management of asthma.

Participation in this study is voluntary and at any time participants are free to withdraw from the study. Families with asthmatic children 5-12 years old suffering with mild asthma are eligible and parent's interested are requested to sign a consent form. Also, parents will be invited to complete a questionnaire regarding socio-demographic information, medical history and dietary habits of their child. As part of the usual medical care, pulmonary function of participating children will be examined using spirometry (which will be free of charge) and blood tests which will be conducted at the start of the study and at the end of 6 months. Blood tests will be performed by trained personnel at the Metabolomic clinic, but will be optional. If at any time during blood tests the participant should feel discomfort or should problems arise, the procedure will be stopped and first aid techniques will be applied by the trained personnel. Eligible children will be randomized into two groups: control and intervention. Children in the intervention group will be required to consume two fatty fish meals per week over a period of 6 months. The control group will follow their usual dietary habits. Cost for biochemical tests have been kindly covered by Dr. Tsoukalas at the **Metabolomic Clinic**.

All data collected will be anonymous, kept confidential, stored at the doctor's clinic and later transferred to La Trobe University archives where they will be used exclusively for the purpose of this study. No personal information will be used for the purpose of this study. Findings will be published in a PhD thesis, journal articles and in presentations. All those involved in this study declare no conflicts of interest.

We kindly thank you for participating and making this study possible. For enquiries please do not hesitate to contact one of the researchers involved in this study.

If you have any complaints or concerns about your participation in the study that the researcher has not been able to answer to your satisfaction, you may contact the Senior Human Ethics Officer, Ethics and Integrity, Research Office, La Trobe University, Victoria, 3086 (Tel:00613 9479 1443, E: humanethics@latrobe.edu.au). Please quote the Ethics application reference number: **HEC: 16-035**.

Research Group

Paediatric Pneumologist : Dr. Ch. Katsardis, 210-9322946,
Principal Supervisor: Dr. C. Itsiopoulos 0061394793640,
Research Dietitian: Maria Papamichael, 6947-073672,

katsardis@yahoo.gr
c.itsiopoulos@latrobe.edu.au
sassipap@hotmail.com

2b) Participant Information Sheet (Greek)



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

Επικ. Καθ. Παιδιατρικής Χ. Κατσαρδής
Πάφου 1, Μαρούσι, Αθήνα, 15125

ΕΝΗΜΕΡΩΤΙΚΟ ΦΥΛΛΑΔΙΟ

Ερευνητική μελέτη με τίτλο «**Η επίδραση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρά ψάρια σε παιδιά που πάσχουν από άσθμα**» θα πραγματοποιηθεί από τη Υπ.Διδάκτωρ του Πανεπιστημίου La Trobe της Μελβούρνης, Μαρία Παπαμιχαήλ, Διαιτολόγο, με υπεύθυνη καθηγήτρια την Δρ. Κ. Ιτσιόπουλος σε συνεργασία με τον παιδοπνευμολόγο Επίκουρο Καθηγητή Παιδιατρικής, Χ Κατσαρδής.

Παγκοσμίως, το άσθμα έχει καταστεί μία από τις πιο συχνές χρόνιες ασθένειες σε παιδιά, η οποία οφείλεται σε γενετικούς και περιβαλλοντικούς παράγοντες. Ωστόσο, σύμφωνα με μελέτες που έχουν γίνει μέχρι σήμερα η μεσογειακού τύπου διατροφή και η κατανάλωση ψαριών σε παιδιά, φαίνεται να δρα προφυλακτικά στην εμφάνιση άσθματος αλλά συσχετίζεται και με μείωση των ήδη υπάρχοντων συμπτωμάτων.

Σκοπός της παρούσας μελέτης είναι, να εξετάσει αν η κατανάλωση λιπαρών ψαριών δρα ευεργετικά στην αναπνευστική λειτουργία, στη μείωση των φαρμάκων, του αριθμού και της σοβαρότητας των ασθματικών κρίσεων σε παιδιά. Τα ευρήματα της μελέτης θα βοηθήσουν στο σχεδιασμό των διατροφικών συστάσεων για τη διαχείριση του άσθματος.

Η συμμετοχή στην έρευνα είναι εθελοντική και δίνεται η δυνατότητα στους συμμετέχοντες να αποσυρθούν από τη μελέτη οποιαδήποτε στιγμή, χωρίς καμία επίπτωση. Στη μελέτη θα συμμετάσχουν παιδιά 5-12 ετών, με ήπιο-μέτριο άσθμα των οποίων οι γονείς θα συναινέσουν υπογράφοντας ένα συμφωνητικό εθελοντικής συμμετοχής. Επίσης θα κληθούν να απαντήσουν σε ερωτηματολόγια που αφορούν στην καταγραφή των δημογραφικών τους στοιχείων, στο ιατρικό ιστορικό, και στις διατροφικές συνήθειες των παιδιών τους. Η αναπνευστική λειτουργία των παιδιών θα εξεταστεί με σπιρομέτρηση και θα διενεργηθούν αιματολογικές εξετάσεις. Η καταγραφή θα πραγματοποιηθεί στην αρχή της μελέτης και μετά από τη πάροδο 6 μηνών. Τα παιδιά υπο μελέτη θα καταταχθούν τυχαία σε μια από τις δύο ομάδες παρέμβασης-ελέγχου. Τα παιδιά που θα συμμετάσχουν στην ομάδα παρέμβασης καλούνται να ακολουθούν Μεσογειακή Διατροφή εμπλουτισμένη με 2 γεύματα/εβδομάδα λιπαρού ψαριού για 6 μήνες. Τα παιδιά που θα συμμετάσχουν στην ομάδα ελέγχου καλούνται να ακολουθούν τη συνήθη διατροφή τους. Οι μετρήσεις σπιρομέτρησης, εξετάσεις αίματος και ούρων στην αρχή της μελέτης αλλά και μετά από έξι μήνες προσφέρονται δωρεάν από την «**Μεταβολομική Κλινική**».

Όλα τα στοιχεία και οι απαντήσεις των ερωτηματολογίων είναι ανωνυμοποιημένα, απόρρητα και θα αποθηκευτούν σε ασφαλή τοποθεσία στο Πανεπιστήμιο La Trobe και τα δεδομένα θα χρησιμοποιηθούν αποκλειστικά και μόνο για τους ερευνητικούς σκοπούς της μελέτης. Τα ευρήματα θα δημοσιευτούν στη διδακτορική διατριβή, επιστημονικά άρθρα και παρουσιάσεις. Όσοι εμπλέκονται σε αυτή τη μελέτη δεν δήλωσαν καμία σύγκρουση συμφερόντων.

Ευχαριστούμε για τη συμμετοχή σας στην πραγματοποίηση αυτής της προσπάθειας.

Για οποιαδήποτε απορία/ διευκρίνιση μη διστάσετε να επικοινωνήσετε με οποιοδήποτε μέλος της ερευνητικής ομάδας ή με το Τμήμα Δεοντολογίας του Πανεπιστημίου La Trobe της Μελβούρνης, τηλ: 00613 94791443.

E-mail: humanethics@latrobe.edu.au. Παρακαλώ να αναφέρετε τον αριθμό αναφοράς της αίτησης βιοηθικής **HEC: 16-035**.

Η Ερευνητική Ομάδα

Ο Παιδοπνευμολόγος, Επικ. Καθηγητής Παιδιατρικής: Χ. Κατσαρδής, 210-9322946, katsardis@yahoo.gr

Η Επιβλέπουσα Καθηγήτρια: Δρ. Κ. Ιτσιόπουλος, 0061394793640, c.itsiopoulos@latrobe.edu.au

Η Ερευνήτρια: Μαρία Παπαμιχαήλ Διαιτολόγος, 6947073672, sassipap@hotmail.com

3a) Consent form



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

Participant ID:

PARTICIPANT CONSENT FORM

I, as the parent/guardian of my child,

I give my child the permission, provided that he/she agrees to participate in the study “The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in asthmatic children” conducted by La Trobe University, Melbourne, Australia.

I declare that I am the person responsible for giving my child permissionto consent in participating in this study.

I understand that the study involves the completion of a questionnaire and consumption of two fish meals per week over a period of 6 months. I have read or have had read to me and understood the participant information statement and consent form, and any questions that I have asked or my child have been answered to our satisfaction. I understand that even though we agree to be involved in this project, he/she can withdraw from the study at any time, and we can withdraw our data up to four weeks following the completion of our participation in the research. Further, in withdrawing from the study, I can request that no information from our involvement be used. I agree that research data provided by us or with our permission during the project may be included in a PhD thesis, presented at conferences and published in journals on the condition that neither our names nor any other identifying information is used.”

Name of Participant (block letters):

Signature:

Date:

Paediatric Pneumologist & co-Supervisor: Dr. Ch. Katsardis
Supervisor: Prof. C. Itsiopoulos
Investigator: Maria. M. Papamichael

Signature:
Signature:
Signature:

3b) Consent form (Greek)



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

ΣΥΓΚΑΤΑΘΕΣΗ ΕΘΕΛΟΝΤΙΚΗΣ ΣΥΜΜΕΤΟΧΗΣ ΣΕ ΕΡΕΥΝΗΤΙΚΗ ΜΕΛΕΤΗ

Ο/Η, ως κηδεμόνας του/της
....., δηλώνω τη συγκατάθεση μου να συμμετάσχει
στην μελέτη «Η θεραπευτική δράση της Μεσογειακής Διατροφής εμπλουτισμένο με λιπαρά ψάρια σε
παιδιά που πάσχουν από άσθμα» που διεξάγεται από το Πανεπιστήμιο του La Trobe της Αυστραλίας.

Δηλώνω ότι είμαι το υπεύθυνο άτομο για να δώσω συγκατάθεση για την συμμετοχή του/της
..... στη μελέτη.

Γνωρίζω ότι η έρευνα συνίσταται στη συμπλήρωση ερωτηματολογίων και στην κατανάλωση δυο γεύμα
των λιπαρών ψαριών ανα εβδομάδα, για 6 μήνες. Οποιαδήποτε πληροφορία ή περαιτέρω διευκρίνιση θα
δοθεί σε μένα και το συμμετέχον τέκνο μου από τους υπευθύνους. Γνωρίζω ότι ο συμμετέχοντας
διατηρεί το δικαίωμα να διακόψει τη συμμετοχή του, όποια στιγμή επιθυμεί χωρίς να έχει κάποια
επίπτωση και χωρίς να απαιτείται να εξηγήσει το λόγο. Επίσης, γνωρίζω ότι όλα τα προσωπικά στοιχεία,
εμού και του τέκνου μου, και τα αποτελέσματα της μελέτης είναι απόρρητα και θα χρησιμοποιηθούν
μόνο για ερευνητικούς σκοπούς.

Έχω διαβάσει το ενημερωτικό φυλλάδιο και υπογράφω με ελεύθερη βούληση.

-----/-----/-----

(υπογραφή γονέα/κηδεμόνα)

Ο Επικ. Καθ. Παιδιατρικής, Χ.Κατσαρδής

Υπογραφή:.....

Η Υπεύθυνη Καθηγήτρια: Δρ.Κ. Ιτσιόπουλος

Υπογραφή:.....

Η Ερευνήτρια : Μαρία Μ. Παπαμιχαήλ

Υπογραφή:.....

Κωδικός Συμμετέχοντας/...../...../.....

4a) Withdrawal form



La Trobe University
Human Ethics Committee
 Melbourne, Australia, 3086.

Withdrawal of Consent for Use of Data Form

Project Title: The prophylactic potential of a Mediterranean dietary pattern enriched with fatty fish in childhood asthma.

I,....., as guardian to my childwish to terminate our participation in this study. In addition I wish to WITHDRAW my consent for the use of all data arising from our participation as mentioned in the information sheet and consent form. Data arising from my participation must NOT be used in this research project as described in the Information and Consent Form. I understand that data arising from my and my child's participation will be destroyed provided that this request is received within four weeks of the completion of my participation in this project. I understand that this withdrawal notification will be retained together with my consent form as evidence of termination of our participation in this study and consent to use the data that we have provided specifically for this research project.

Participant's name (printed):

.....

Signature:

.....

Date:....\....\.....

4b) Withdrawal form (Greek)



La Trobe University
Human Ethics Committee
 Melbourne, Australia, 3086

**ΑΙΤΗΣΗ ΔΙΑΚΟΠΗΣ ΤΗΣ ΣΥΜΜΕΤΟΧΗΣ ΚΑΙ ΧΡΗΣΗ ΤΩΝ ΠΡΟΣΩΠΙΚΩΝ
 ΔΕΔΩΜΕΝΩΝ**

Τίτλο μελέτης: Η θεραπευτική δράση της Μεσογειακής διατροφής σε παιδιά που πάσχουν από άσθμα.

Ο/Η, ως κηδεμόνας του/της δηλώνω το δικαίωμα μας **ΝΑ ΔΙΑΚΟΨΟΥΜΕ** την συμμετοχή μας. Επίσης επιθυμώ **ΝΑ ΑΠΟΣΥΡΘΟΥΝ** όλα τα προσωπικά στοιχεία, εμού και του τέκνου μου, και τα δεδομένα μας όπως αναφέρεται στο ενημερωτικό φυλλάδιο και στο Συμφωνητικό Εθελοντικής Συμμετοχής. Γνωρίζω ότι όλα τα προσωπικά μου δεδομένα και του τέκνου μου θα καταστραφούν εφόσον η κατάθεση της αίτησής γίνεται εντός τεσσάρων εβδομάδων από την ολοκλήρωση της μελέτης. Γνωρίζω ότι ή αίτηση μου για την διακοπή της συμμετοχής θα κρατηθεί μαζί με το συμφωνητικό εθελοντικής συμμετοχής ως απόδειξη διακοπής της συνεργασίας μας και της χρήσης των δεδομένων μας στην μελέτη αυτή.

Όνομα Συμμετέχοντα:

Υπογραφή:

Ημερομηνία:\.....\.....

5a) Screening Questionnaire



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ΣΚΛΑΒΕΝΙΤΗΣ

Date:/..... /

Screening Questionnaire

Completed by: interviewer

Refers to: Children 5-12 years

Respondents: Parents/Carers

Respondent's Details:

Name:

SCREENING CRITERIA

Ep.1. How old is your child? (5-12 years old):/...../.....

Was your child born between **2004** and **2011**? YES ☐ NO ☐

Ep.2. Does your child have mild-intermittent asthma? YES ☐ NO ☐

If the answer to the above two questions is YES, then continue with the **EXCLUSION CRITERIA**.

However, if the answer to at least one of the two questions is NO, then the child does not qualify to participate in this study.

EXCLUSION CRITERIA

Ep.3. Perhaps your child does not eat the following fatty fish: sardines, salmon, trout, anchovies, gilthead sea bream, chubb mackerel, mackerel?

YES, he does not eat ☐ NO, he eats ☐

If he doesn't eat fatty fish, why?

He is allergic ☐ He/she does not like to eat this kind of fish ☐ He/she is vegetarian ☐

Ep.4. Does your child suffer from any of the following medical conditions?

GERD YES ☐ NO ☐

Cystic Fibrosis YES ☐ NO ☐

Congenital Pulmonary Airway Disease YES ☐ NO ☐

Ep.5. Does your child take fish oil supplements?

YES ☐ NO ☐

If the answer to at least one of the above questions of the exclusion criteria (Q3-Q5) is YES, then your child is **not eligible to participate in this study**. On the other hand, if the response to ALL of the questions of the exclusion criteria is **NO**, then your child is **ELIGIBLE** to participate. If the parent/carer of the eligible child agrees and signs the consent form, then the child becomes a **PARTICIPANT** in this study and will be assigned a participant identification code (ID).

5b) Screening Questionnaire (Greek)



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ΣΚΛΑΒΕΝΙΤΗΣ

Ημερομηνία:/...../.....

Ερωτηματολόγιο Επιλεξιμότητας (Screening)

Συμπληρώνεται από τον: συνεντευκτή

Αφορά: Παιδιά 5-12 ετών

Απαντούν: Γονείς/κηδεμόνες

Στοιχεία Γονέα/κηδεμόνα που απαντά στις ερωτήσεις

Όνομα:.....

ΚΡΙΤΗΡΙΑ ΕΙΣΑΓΩΓΗΣ**Ερ.1. Πότε γεννήθηκε το παιδί σας (5-12 ετών):/...../.....**Έχει γεννηθεί μεταξύ **2004** και **2011**; ΝΑΙ ☐ ΟΧΙ ☐**Ερ.2. Έχει ήπιο-μέτριο άσθμα;** ΝΑΙ ☐ ΟΧΙ ☐

Αν η απάντηση είναι ΝΑΙ και στις δύο ερωτήσεις τότε θα πρέπει να ελεγχθούν και τα ΚΡΙΤΗΡΙΑ ΑΠΟΚΛΕΙΣΜΟΥ. Αν έστω σε μια από τις δύο ερωτήσεις η απάντηση είναι ΟΧΙ τότε το παιδί δεν έχει δικαίωμα συμμετοχής στη μελέτη.

ΚΡΙΤΗΡΙΑ ΑΠΟΚΛΕΙΣΜΟΥ**Ερ.3. Μήπως το παιδί σας δεν τρώει κανένα από τα εξής λιπαρά ψάρια: σαρδέλα, σολομό, γαύρο, τσιπούρα, κολιό, πέστροφα, σκουμπρί;**ΝΑΙ, δεν τρώει ☐ ΟΧΙ τρώει ☐

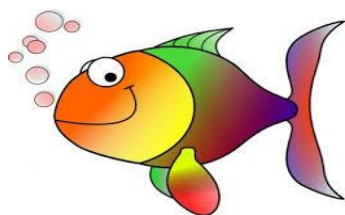
Αν, δεν τρώει γιατί;

Είναι αλλεργικό ☐ Δεν του αρέσει κανένα από τα λιπαρά ψάρια ☐ Είναι χορτοφάγο ☐**Ερ.4. Μήπως το παιδί σας έχει κάποιο από τα παρακάτω ιατρικά προβλήματα;**Γαστροφαγική Παλινδρόμηση ΝΑΙ ☐ ΟΧΙ ☐Κυστική Ίνωση ΝΑΙ ☐ ΟΧΙ ☐Συγγενή Ανωμαλία Αναπνευστικού ΝΑΙ ☐ ΟΧΙ ☐**Ερ.5. Μήπως το παιδί σας παίρνει ιχθυέλαιο συμπλήρωμα;**ΝΑΙ ☐ ΟΧΙ ☐

Αν η απάντηση τουλάχιστον σε μια από τις παραπάνω ερωτήσεις των ΚΡΙΤΗΡΙΩΝ ΑΠΟΚΛΕΙΣΜΟΥ (Ερ.3-Ερ.5) είναι ΝΑΙ τότε το παιδί αποκλείεται από τη μελέτη. Αν σε όλες τις παραπάνω ερωτήσεις των κριτηρίων αποκλεισμού η απάντηση είναι ΟΧΙ τότε το παιδί είναι ΕΠΙΛΕΞΙΜΟ (έχει δικαίωμα συμμετοχής).

Αν για το ΕΠΙΛΕΞΙΜΟ παιδί υπογραφεί η φόρμα συγκατάθεσης τότε είναι ΣΥΜΜΕΤΕΧΟΝΤΑΣ και πρέπει να λάβει κωδικό συμμετοχής.

6a) Socio-demographic Questionnaire baseline



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IDENTIFICATION AND CONTACT DETAILS

Date: / /

Participant's ID: / /

Child's Name:	Age: years
Home Address:	
Suburb:	
Postcode:	
Parent's e-mail:	
Respondent's Telephone (Home) :	
Respondent's Telephone (Mob):	

Socio-demographic Questionnaire

Completed by: parent/guardian

Refers to: Children 5-12 years

Respondents: Parents/ or guardians

Details of Parents/or guardian completing this questionnaire

Name of respondent:.....

Q.1. What is your relationship with the child?

Mother ☐ Grandfather ☐
 Father ☐ Grandmother ☐
 Guardian ☐

Q.2. What nationality are you?

Greek ☐ Other ☐

Q.3. What race do you belong to?

Caucasian ☐ Black/or African ☐ Asian ☐

Q.4. What is your marital status? Are you.....

Single ☐
 Married ☐
 Widowed ☐
 Divorced ☐
 Separated ☐
 Living together ☐

Q.5. What is your current employment status? Are you.....

- | | |
|---|--------------------------|
| Unemployed | <input type="checkbox"/> |
| Working Full-time | <input type="checkbox"/> |
| Working Part-time | <input type="checkbox"/> |
| Self-Employed | <input type="checkbox"/> |
| Working in Public Sector | <input type="checkbox"/> |
| Working in Private Sector | <input type="checkbox"/> |
| Retired | <input type="checkbox"/> |
| Put off work or closed business | <input type="checkbox"/> |
| House-wife or baby sitter | <input type="checkbox"/> |
| Unable to work or permanently handicapped | <input type="checkbox"/> |
| Other case unable to work | <input type="checkbox"/> |

Q.6. What education level have you completed?

Primary School	<input type="checkbox"/>	Technical College	<input type="checkbox"/>
Junior High	<input type="checkbox"/>	University	<input type="checkbox"/>
Senior High	<input type="checkbox"/>	Masters	<input type="checkbox"/>
College	<input type="checkbox"/>	PhD	<input type="checkbox"/>

Details of spouse**Q.7. What nationality is your spouse?**

Greek ☐ Other ☐

Q.8. What race does your spouse belong to?

Caucasian ☐ Black/or African ☐ Asian ☐

Q.9. What is your spouse current employment status?

Unemployed ☐

Working Full-time ☐

Working Part-time ☐

Self-Employed ☐

Working in Public Sector ☐

- Working in Private Sector ☐
- Retired ☐
- Put off work or closed business ☐
- House-wife or baby sitter ☐
- Unable to work or permanently handicapped ☐
- Other reason ☐

Q.10. What education level has your spouse completed?

- | | |
|---|--|
| Primary School <input type="checkbox"/> | Technical College <input type="checkbox"/> |
| Junior High <input type="checkbox"/> | University <input type="checkbox"/> |
| Senior High <input type="checkbox"/> | Masters <input type="checkbox"/> |
| College <input type="checkbox"/> | PhD <input type="checkbox"/> |

Q.11. What is your family's monthly income?

(Include total income not only due to employment and rent)

.....

Child's Details

Q.12. How many children are in the family?

Number of children in the family

Q.13. What ranking is the participating child in your family?

First ☐ Second ☐ Third ☐ Fourth ☐

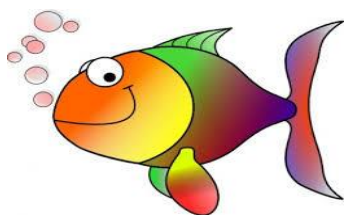
Q.14. What sex is the participating child? Boy ☐ Girl ☐

Q.15. What is the participant's date of birth? ____/____/____

Q.16. What weight and height is your child today? kg cm

Q.17. What type of school does the participant attend? Private ☐ Public ☐

6b) Socio-demographic questionnaire baseline (Greek)



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ΣΚΛΑΒΕΝΙΤΗΣ

Στοιχεία Ταυτότητας –Επικοινωνίας

Ημερομηνία : / /

Κωδικός συμμετέχοντα:/...../.....

Όνομα παιδιού :	Ηλικία:	ετών
Διεύθυνση:		
Περιοχή:		
Ταχυδρομικό κώδικας:		
E-mail Γονέα:		
Τηλέφωνο αποκριμένου γονέα (σταθερό):		
Τηλέφωνο αποκριμένου γονέα (κινητό):		

Ερωτηματολόγιο Δημογραφικών και Κοινωνικοοικονομικών Χαρακτηριστικών

Συμπληρώνεται από τον: συνεντευκτή/ γονέα

Αφορά: Παιδιά 5-12 ετών

Απαντούν: Γονείς/κηδεμόνες

Στοιχεία Γονέα/κηδεμόνα που απαντά στις ερωτήσεις

Όνομα:.....

Ερ1. Τι σχέση έχετε με το παιδί;Μητέρα ☐Παππούς ☐Πατέρας ☐Γιαγιά ☐Κηδεμόνας ☐**Ερ2. Τι εθνικότητα έχετε;**Ελληνική ☐Άλλη ☐**Ερ3. Σε ποιά φυλή θα κατατάσσετε τον εαυτό σας;**Καυκάσιος/α ☐Μαύρος/η/Αφρικανή ☐Ασιάτης/α ☐**Ερ4. Ποιά είναι η οικογενειακή σας κατάσταση; Είστε:**Άγαμος (δεν έχετε παντρευτεί) ☐Έγγαμος (είστε παντρεμένος/η) ☐Χήρος/χήρα ☐Διαζευγμένος (έχει εκδοθεί το διαζύγιο) ☐Σε διάσταση ☐Με σύμφωνο συμβίωσης ☐

Ερ 5. Με τι ασχολείστε σήμερα; Είστε;

- Άνεργος ☐
- Μισθωτός με πλήρη απασχόληση ☐
- Μισθωτός με μερική απασχόληση ☐
- Ελεύθερος επαγγελματίας ☐
- Δημόσιος υπάλληλος ☐
- Ιδιωτικός υπάλληλος ☐
- Συνταξιούχος ☐
- Έχετε διακόψει την εργασία ή την επιχείρησή σας ☐
- Νοικοκυρά ή φροντίδα παιδιών ☐
- Ακατάλληλος για εργασία ή έχετε μόνιμη αναπηρία ☐

Ερ 6. Τι επίπεδο εκπαίδευσης έχετε τελειώσει;

- | | | | |
|--------------|--------------------------|--------------|--------------------------|
| Δημοτικό | <input type="checkbox"/> | ΤΕΙ | <input type="checkbox"/> |
| Γυμνάσιο | <input type="checkbox"/> | ΑΕΙ | <input type="checkbox"/> |
| Λύκειο | <input type="checkbox"/> | Μεταπτυχιακό | <input type="checkbox"/> |
| Κολλέγιο-ΙΕΚ | <input type="checkbox"/> | Διδακτορικό | <input type="checkbox"/> |

Στοιχεία Συζύγου**Ερ 7. Ο/Η σύζυγός σας τι εθνικότητα έχει;**Ελληνική ☐ Άλλη ☐**Ερ 8. Σε ποιά φυλή θα κατατάσσατε τον/την σύζυγό σας;**Καυκάσιος/α ☐ Μαύρος/η/Αφρικανή ☐ Ασιάτης/α ☐**Ερ 9. Ο/Η σύζυγός σας με τι ασχολείται σήμερα; Είναι;**Άνεργος ☐Μισθωτός με πλήρη απασχόληση ☐Μισθωτός με μερική απασχόληση ☐Ελεύθερος επαγγελματίας ☐Δημόσιος υπάλληλος ☐Ιδιωτικός υπάλληλος ☐Συνταξιούχος ☐Έχετε διακόψει την εργασία ή την επιχείρησή σας ☐Νοικοκυρά ή φροντίδα παιδιών ☐Ακατάλληλος για εργασία ή έχετε μόνιμη αναπηρία ☐Άλλη περίπτωση μη οικονομικού ανέργου ατόμου ☐

Ερ 10. Ο/Η σύζυγός σας τι επίπεδο εκπαίδευσης έχει τελειώσει;

Δημοτικό	<input type="checkbox"/>	ΤΕΙ	<input type="checkbox"/>
Γυμνάσιο	<input type="checkbox"/>	ΑΕΙ	<input type="checkbox"/>
Λύκειο	<input type="checkbox"/>	Μεταπτυχιακό	<input type="checkbox"/>
Κολλέγιο-ΙΕΚ	<input type="checkbox"/>	Διδακτορικό	<input type="checkbox"/>

Ερ 11. Ποιο είναι το οικογενειακό μηναίο εισόδημα σας; (αφορά στο συνολικό εισόδημα, όχι μόνο λόγω εργασίας και ενοίκια;

.....

Στοιχεία Παιδιού

Ερ 12. Πόσα παιδιά είναι συνολικά στην οικογένεια;

Αριθμός παιδιών στην οικογένεια

Ερ 13. Ποιά είναι η κατάταξη του συμμετέχοντος παιδιού στην οικογένεια;

Πρώτο ☐ Δεύτερο ☐ Τρίτο ☐ Τέταρτο ☐

Ερ 14. Ποιο είναι το φύλο του συμμετέχοντος παιδιού; Αγόρι ☐ Κορίτσι ☐

Ερ 15. Ποια είναι η ημερομηνία γεννήσεως του συμμετέχοντος παιδιού; ____/____/____

Ερ 16. Τι βάρος και ύψος έχει το παιδί σας;Κιλά εκ.

Ερ 17. Σε τι είδος σχολείο πηγαίνει το συμμετέχον παιδί; Ιδιωτικό ☐ Δημόσιο ☐

7a) Medical Questionnaire



Participant's ID: /...../.....

Medical Questionnaire

Completed by Interviewer

Refers to: Parents/guardians

Respondents: Parents/guardians

Details of respondents (parents/guardians)

Name:

Child's name:

Age

Group

Mother's Details

Q.1. Did you smoke during pregnancy? YES ☐ NO ☐

Q.2. Did you smoke during the first year of your child's life? YES ☐ NO ☐

Q.3. Do you currently smoke? YES ☐ NO ☐

If YES, what brand of cigarettes?
.....

If YES, how many cigarettes per day ☐ or packets of cigarettes per day ☐?

Q.4. During childhood (0-16 years old), did you suffer from ...? YES ☐ NO ☐

Asthma ☐ Rhinitis (Hay fever) ☐ Eczema ☐

Q.5. During adulthood (from 16 years onwards till today) do you suffer from ..? YES ☐ NO ☐

Asthma ☐ Rhinitis (Hay fever) ☐ Eczema ☐

Father's Details

Q.6. Did the father smoke during the first year of the child's life? YES ☐ NO ☐

Q.7. Does the father smoke today? YES ☐ NO ☐

If YES, what brand of cigarettes?
.....

If YES, how many cigarettes per day? ☐ packets of cigarettes per day ☐

Q.8. During childhood (0-16 years), did the father suffer from.... YES ☐ NO ☐

Asthma ☐ Rhinitis (Hay fever) ☐ Eczema ☐

Q.9. During adulthood (from 16 years old till today) did the father suffer from...?YES ☐ NO ☐

Asthma ☐

Rhinitis (Hay fever) ☐

Eczema ☐

The following questions refer to pregnancy and lactation details and the child's medical history

Participant's Details

Q.10. How many weeks was the term of your pregnancy?

32-37 weeks ☐

37-40 weeks ☐

>40 weeks ☐

Q.11. How much did your child weigh at birth?

< 2500 grams ☐

2500- 4000 grams ☐

>4000 grams ☐

Q.12. Was your child born by.....?

Vaginal delivery ☐

Caesarean-section ☐

Q.13. Did you breast-feed your child? YES ☐ NO ☐

If YES, for how long?

<3 months ☐

3-6 months ☐

6-12 months ☐

>12 months ☐

Q.14. At what age was your child diagnosed with asthma?years

Q.15. Does your child suffer from any other allergies? YES ☐ NO ☐

If YES, does he suffer from

Rhinitis/or Hay fever ☐

Conjunctivitis ☐

Eczema ☐

Food Allergy ☐ If, YES, what?

Q.16. During the last month has your child taken medication YES ☐ NO ☐

If YES, when.....?

As part of a daily therapy ☐

For how long?weeks




Only as needed during episodes ☐








How many episodes did he/she have?times






When he/she is sick ☐


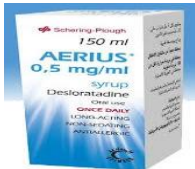



How many times was he/she sick? times

Q.17. What medication does your child take and dosage?

MEDICATION TYPE			DOSAGE
Bronchodilators			
Aerolin (100 µg)		<input type="checkbox"/>times/day
Anti-inflammatory corticosteroids			
Flixotide (125 µg)		<input type="checkbox"/>times/day
Flixotide (250 µg)		<input type="checkbox"/>times/day

MEDICATION TYPE			DOSAGE
Seretide (125 µg)		<input type="checkbox"/>times/day
Seretide (250µg)		<input type="checkbox"/>times/day
Seretide (Discus) (100µg)		<input type="checkbox"/>times/day
Seretide (Discus) (250µg)		<input type="checkbox"/>times/day
Symbicort (80µg)		<input type="checkbox"/>times/day
Symbicort (160µg)		<input type="checkbox"/>times/day
Anti-Leukotrienes			
Singulair (Montelukast) (5mg)		<input type="checkbox"/>times/day

MEDICATION TYPE			DOSAGE
Miralust (Montelukast) (5mg)		<input type="checkbox"/>times/day
Apilone (Montelukast) (5mg)		<input type="checkbox"/>times/day
Modulair (Montelukast) (5mg)		<input type="checkbox"/>times/day
Cortisone (per os)			
Medrol per os (16mg) (Prednisolone)		<input type="checkbox"/>times/day
Prezolon (5mg)		<input type="checkbox"/>times/day
Soldesanil drops		<input type="checkbox"/>times/day
RHINITIS			Dosage
Nasonex		<input type="checkbox"/>times/day
Mometasone nasal spray		<input type="checkbox"/>times/day

MEDICATION TYPE			DOSAGE
Pulmicort nasal		<input type="checkbox"/>times/day
ANTI-HISTAMINES			
AERIUS (syrup)		<input type="checkbox"/>times/day
AERIUS (tablets)			
XYZAL (syrup)		<input type="checkbox"/>times/day
XYZAL (tablets)			

Q18. Does your child take

Nutritional supplements ☐

Vitamins ☐

None of the two ☐

If YES, which one?

How many tablets per day?

7b) Medical Questionnaire (Greek)



METABOLOMIC MEDICINE®
HEALTH CLINICS FOR AUTOIMMUNE AND CHRONIC DISEASES



Βασιλόπουλος
...και του πουλιού το γάλα!



Κωδικός συμμετέχοντα:/...../.....

Ερωτηματολόγιο Ιατρικών Πληροφοριών

Συμπληρώνεται από τον: συνεντευκτή/ γονέα

Αφορά: Γονείς/κηδεμόνες και παιδιά

Απαντούν: Γονείς/κηδεμόνες

Στοιχεία Γονέα/κηδεμόνα που απαντά στις ερωτήσεις

Όνομα:.....

Όνομα παιδιού

Ηλικία

Ομάδα

Πληροφορίες για τη μητέρα

Ερ. 1. Καπνίζατε κατά τη διάρκεια της εγκυμοσύνης; NAI ☐ OXI ☐

Ερ. 2. Καπνίζατε κατά τη διάρκεια του πρώτου έτους της ζωής του παιδιού; NAI ☐ OXI ☐

Ερ. 3 Καπνίζετε σήμερα; NAI ☐ OXI ☐

Αν NAI, ποια μάρκα;

.....

Αν NAI, πόσα τσιγάρα/ημέρα; ☐ ή πόσα πακέτα/ημέρα ☐ ;

Ερ. 4. Όταν ήσασταν μικρή (κάτω από 16 ετών) είχατε: NAI ☐ OXI ☐

Άσθμα; ☐

Ρινίτιδα; ☐

Έκζεμα; ☐

Ερ. 5. Στην ενήλικη ζωή (16 ετών ως σήμερα) είχατε: NAI ☐ OXI ☐

Άσθμα; ☐

Ρινίτιδα; ☐

Έκζεμα; ☐

Πληροφορίες για τον πατέρα

Ερ. 6. Ο πατέρας κάπνιζε κατά τη διάρκεια του πρώτου έτους της ζωής του παιδιού;

NAI ☐ OXI ☐

Ερ. 7. πατέρας καπνίζει σήμερα; NAI ☐ OXI ☐

Αν NAI, ποια μάρκα;

.....

Αν NAI, πόσα τσιγάρα/ημέρα; ☐ ή πόσα πακέτα/ημέρα ☐ ;

Ερ. 8. Ο πατέρας όταν ήταν μικρός (κάτω από 16 ετών) είχε: NAI ☐ OXI ☐

Άσθμα; ☐

Ρινίτιδα; ☐

Έκζεμα

Ερ. 9. Ο πατέρας στην ενήλικη ζωή (16 ετών ως σήμερα) έχει: ΝΑΙ ☐ ΟΧΙ ☐

Άσθμα; ☐

Ρινίτιδα; ☐

Έκζεμα; ☐

Πληροφορίες για παιδιά

Ερ. 10. Πόσες εβδομάδες διήρκησε η κύηση μέχρι τον τοκετό;

32-38 βδομάδες ☐

37-40 εβδομάδες ☐

>40 εβδομάδες ☐

Ερ. 11. Τι βάρος είχε το παιδί όταν γεννήθηκε;

< 2500 γραμμάρια ☐

2500- 4000 γραμμάρια ☐

>4000 γραμμάρια ☐

Ερ. 12. Το παιδί γεννήθηκε με:

Φυσιολογικό τοκετό ☐

Καισαρική τομή ☐

Ερ. 13. Θηλάσατε το παιδί όταν ήταν μωρό; ΝΑΙ ☐ ΟΧΙ ☐ Αν ΝΑΙ, για πόσο καιρό;

<3 μήνες ☐

3-6 μήνες ☐

6-12 μήνες ☐

>12 μήνες ☐

Ερ. 14. Σε ποια ηλικία εμφανίστηκε το άσθμα του παιδιού;ετών

Ερ. 15. Το παιδί πάσχει από άλλες αλλεργίες; ΝΑΙ ☐ ΟΧΙ ☐

Αν ΝΑΙ, τότε πάσχει από

Ρινίτιδα/ εποχιακή ρινίτιδα; ☐

Επιπεφυκίτιδα; ☐

Έκζεμα; ☐

Τροφική αλλεργία; ☐

Αν, Ναι σε τι;

Ερ. 16. Το παιδί τον τελευταίο μήνα βρίσκεται σε φαρμακευτική αγωγή; ΝΑΙ ☐ ΟΧΙ ☐




Αν ΝΑΙ, κάθε πότε;

Καθημερινά βάση θεραπείας; ☐ Για πόσο καιρό;εβδομάδες




Μόνο όταν έχει επεισόδιο; ☐ Πόσες φορές είχε επεισόδια; φορές



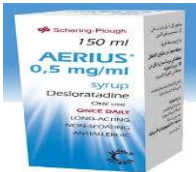

Όταν είναι άρρωστος; ☐ Πόσες φορές ήταν άρρωστος;φορές



Ερ. 17. Τι φάρμακα λαμβάνει και με ποια συχνότητα;

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
ΒΡΟΓΧΟΔΙΑΣΤΑΛΤΙΚΑ			
Aerolin (100 µg)		<input type="checkbox"/>φορές/ημέρα
ΑΝΤΙΦΛΕΓΜΟΝΩΔΗ ΚΟΡΤΙΚΟΕΙΔΗ			
Flixotide (125 µg)		<input type="checkbox"/>φορές/ημέρα
Flixotide (250 µg)		<input type="checkbox"/>φορές/ημέρα
Seretide (125 µg)		<input type="checkbox"/>φορές/ημέρα

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
			
Seretide (250μg)		<input type="checkbox"/>φορές/ημέρα
Seretide (Discus) (100μg)		<input type="checkbox"/>φορές/ημέρα
Seretide (Discus) (250μg)		<input type="checkbox"/>φορές/ημέρα
Symbicort (80μg)		<input type="checkbox"/>φορές/ημέρα
Symbicort (160μg)		<input type="checkbox"/>φορές/ημέρα
ΑΝΤΙΑΕΥΚΟΤΡΙΕΝΕΣ			

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
Singulair (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
Miralust (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
Apilone (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
Modulair (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
KOPTIZONH (per os)			ΔΟΣΟΛΟΓΙΑ
Medrol per os (16mg) (Prednisolone)		<input type="checkbox"/>φορές/ημέρα
Prezolon (5mg)		<input type="checkbox"/>φορές/ημέρα
Soldesanil σταγόνες		<input type="checkbox"/>φορές/ημέρα

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
PINITΙΔΑ			ΔΟΣΟΛΟΓΙΑ
Nasonex		<input type="checkbox"/>φορές/ημέρα
Mometasone nasal spray		<input type="checkbox"/>φορές/ημέρα
Pulmicort nasal		<input type="checkbox"/>φορές/ημέρα
ANTI-ΙΣΤΑΜΙΝΙΚΑ			
Aerius (σιρόπι)		<input type="checkbox"/>φορές/ημέρα
Aerius (ταμπλέτες)			

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
Χοζαλ (σιρόπι)		<input type="checkbox"/>φορές/ημέρα
Χοζαλ (ταμπλέτες)			

Ερ. 18. Το παιδί λαμβάνει ;

Συμπληρώματα διατροφής; ☐ Βιταμίνες; ☐ Τίποτα από τα δύο; ☐

Αν ΝΑΙ, ποιο ; πόσα χάπια/ημέρα.....

8a) Asthma Control Questionnaire



Participant's ID: /...../.....

Questionnaire

Please complete the following questionnaire

Completed by: Parent/guardian

Refers: Parents/guardian and child

Respondents: Parents/Guardians

Details of respondents (parents/guardians)

Name:

Name of participating child:

Group:

Date of birth:

Age:

Asthma Control Questionnaire

This questionnaire is to be completed by the child together with the parent/guardian.

Think how the child's asthma was **during the PAST WEEK**, and mark with a **circle** the response which best describes your condition.

Q1. During the past week, how often were you woken by your asthma during the night?

- 0 Never
- 1 Hardly ever
- 2 A few times
- 3 Several times
- 4 Many times
- 5 A great many times
- 6 Unable to sleep because of asthma

Q2. During the past week, how bad were your symptoms (e.g hard to breathe, wheeze, cough) when you woke up in the morning?

- 0 No symptoms
- 1 Very mild symptoms
- 2 Mild symptoms
- 3 Moderate symptoms
- 4 Quite severe symptoms
- 5 Severe symptoms
- 6 Very severe symptoms

Q3. During the past week, how limited were you in your activities because of your asthma (e.g absent from school or lessons)?

- 0 Not limited at all
- 1 Very slightly limited
- 2 Slightly limited
- 3 Moderately limited
- 4 Very limited
- 5 Extremely limited
- 6 Totally limited

Q4. During the past week, how much shortness of breathe did you experience because of your asthma?

- 0 None
- 1 A very little
- 2 A little
- 3 A moderate amount
- 4 Quite a lot
- 5 A great deal
- 6 A very great deal

Q5. During the past week, how much of the time did you wheeze?

- 0 Never
- 1 Hardly any of the time
- 2 A little of the time
- 3 A moderate amount of the time
- 4 A lot of the time
- 5 Most of the time
- 6 All of the time

Q6. During the past week, how many puff/inhalations of your reliever have you used each day? (e.g Aerolin / Serevent)?

(If you are not sure how to answer this question please ask for assistance)

- 0 None
- 1 1-2 puffs/inhalations most days
- 1 3-4 puffs/inhalations most days
- 3 5-8 puffs/inhalations most days
- 4 9-12 puffs/inhalations most days
- 5 13-16 puffs/inhalations most days
- 6 More than 16 puffs/inhalations most days

Q7a. FEV₁ predicted (completed by candidate)

pre-bronchodilator: FEV₁ %predicted (value).....
Score.....

Score % predicted FEV ₁ :		0	> 95% predicted	
1	95-90%	2	89-80%	3 79-70%
4	69-60%	5	59-50%	6 <50% predicted

Q7b. Since your last check up at the asthma clinic, did you have an unexpected visit to the hospital or to emergency admissions or to the asthma specialist?

YES ☐

NO ☐

IF YES, how many times

8b) Asthma Control Questionnaire (Greek)



Κωδικός συμμετέχοντα:/...../.....

Ερωτηματολόγιο

Παρακαλώ συμπληρώστε το ερωτηματολόγιο.

Συμπληρώνεται από τον: συνεντευκτή/ γονέα

Αφορά: Γονείς/κηδεμόνες και παιδιά

Απαντούν: Γονείς/κηδεμόνες

Στοιχεία Γονέα/κηδεμόνα που απαντά στις ερωτήσεις

Όνομα:

Όνομα παιδιού

Ομάδα

Ημερομηνία γεννήσεως:

Ηλικία:.....

Ερωτηματολόγιο Παιδιών για τον έλεγχο του ‘Άσθματος

Αυτό το ερωτηματολόγιο να συμπληρωθεί από το παιδί μαζί με τον γονέα/κηδεμόνα
Σκεφθείτε πως ήσασταν την **ΠΕΡΑΣΜΕΝΗ ΕΒΔΟΜΑΔΑ**, και βάλτε **σε κύκλο** τον αριθμό της
απάντησης που περιγράφει καλύτερα την κατάσταση σας.

**Ερ.1. Γενικά, την περασμένη εβδομάδα,
πόσες φορές ξυπνούσατε από το άσθμα σας
μέσα στη νύχτα;**

- 0 Ποτέ
- 1 Σχεδόν ποτέ
- 2 Λίγες φορές
- 3 Αρκετές φορές
- 4 Πολλές φορές
- 5 Πάρα πολλές φορές
- 6 Δεν μπορούσα να κοιμηθώ λόγω του άσθματος

**Ερ.2. Γενικά, την περασμένη εβδομάδα, πόσο άσχημα
ήταν τα συμπτώματα του άσθματος σας όταν
ξυπνούσατε το πρωί (π.χ δύσπνοια, βήχα,
σφύριγμα);**

- 0 Κανένα σύμπτωμα
- 1 Πολύ ελαφρά συμπτώματα
- 2 Ελαφρά συμπτώματα
- 3 Μέτρια συμπτώματα
- 4 Αρκετά σοβαρά συμπτώματα
- 5 Σοβαρά συμπτώματα
- 6 Πολύ σοβαρά συμπτώματα

**Ερ.3.Γενικά, την περασμένη εβδομάδα, πόσο
περιορισμένες ήταν οι δραστηριότητες σας λόγω του άσθματος
σας (π.χ απουσία από το σχολείο/ ή μάθημα);**

- 0 Καθόλου περιορισμένες
- 1 Πολύ λίγο περιορισμένες
- 2 Λίγο περιορισμένες
- 3 Μέτρια περιορισμένες
- 4 Πολύ περιορισμένες
- 5 Υπερβολικά περιορισμένες
- 6 Τελείως περιορισμένες

Ερ.4. Γενικά, την περασμένη εβδομάδα, πόσο λαχάνιασμα νιώσατε λόγω του άσθματός σας;

- 0 Καθόλου
- 1 Πολύ λίγο
- 2 Λίγο
- 3 Μέτριο
- 4 Αρκετό
- 5 Πολύ
- 6 Πάρα πολύ

Ερ.5. Γενικά, την περασμένη εβδομάδα, πόσο χρόνο είχατε σφύριγμα στο στήθος;

- 0 Ποτέ
- 1 Σχεδόν ποτέ
- 2 Λίγο από το χρόνο
- 3 Μέτριο από το χρόνο
- 4 Αρκετό από το χρόνο
- 5 Τον περισσότερο χρόνο
- 6 Συνέχεια

Ερ.6. Γενικά, την περασμένη εβδομάδα, πόσες εισπνοές κάνατε κάθε μέρα από το φάρμακο για γρήγορη ανακούφιση (π.χ Aerolin / Serevent);

(Αν δεν είσατε σίγουρος/η πώς να απαντήσετε αυτή της ερώτηση, παρακαλούμε ζητήστε βοήθεια)

- 0 Καμιά
- 1 1-2 εισπνοές τις περισσότερες μέρες
- 1 3-4 εισπνοές τις περισσότερες μέρες
- 3 5-8 εισπνοές τις περισσότερες μέρες
- 4 9-12 εισπνοές τις περισσότερες μέρες
- 5 13-16 εισπνοές τις περισσότερες μέρες
- 6 Πάνω από 16 εισπνοές τις περισσότερες

Ερ.7a. FEV₁ predicted pre-bronchodilator: FEV₁ %predicted (τιμή).....

Σκόρ

Ερ.7b. Από την τελευταία σας επίσκεψη στο ιατρό, είχατε καμία προγραμματίστη επίσκεψη στον ιατρό, ή στο νοσοκομείο στα επείγοντα περιστατικά;

NAI ☐ OXI ☐

Αν, NAI πόσες φορές

9a) Paediatric Asthma Quality of Life Questionnaire

MINI PAEDIATRIC ASTHMA QUALITY OF LIFE QUESTIONNAIRE

Please complete all questions by circling the number that best describes how you have been during the last week as a result of your asthma.

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK BY.....?

	Extremel y bothered	Very bothere d	Quite bothered	Somewhat bothered	Bothered a bit	Hardly bothered at all	Not bothered
Q.8. COUGHING	1	2	3	4	5	6	7
Q.9. WHEEZING	1	2	3	4	5	6	7
Q.10. TIGHTNESS IN CHEST	1	2	3	4	5	6	7
IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU?							
Q.11. Feel OUT OF BREATHE?	1	2	3	4	5	6	7
Q.12. Feel TIRED because of your asthma?	1	2	3	4	5	6	7
Q.13. Have trouble sleeping AT NIGHT because of your asthma?	1	2	3	4	5	6	7
Q.14. Feel FRUSTRATED because of your asthma?							
Q.15. Feel FRIGHTENED OR WORRIED because of your asthma?	1	2	3	4	5	6	7
Q.16. Feel IRRITABLE (cranky/grouchy) because of your asthma?	1	2	3	4	5	6	7
Q.17. Feel DIFFERENT or LEFT OUT because of your asthma?	1	2	3	4	5	6	7
HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK DOING?							
Q.18. PHYSICAL ACTIVITIES (such as running, swimming, uphill/upstairs and cycling)?	1	2	3	4	5	6	7
Q.19. BEING WITH ANIMALS (such as playing with pets and looking after animals)?	1	2	3	4	5	6	7
Q.20. ACTIVITIES WITH FRIENDS AND FAMILY (Such as playing at recess and doing things with your friends and family)?	1	2	3	4	5	6	7

9b) Paediatric Asthma Quality of Life Questionnaire (Greek)

Μίνι Ερωτηματολόγιο Ποιότητας Ζωής του Παιδικού Άσθματος

Αυτό το ερωτηματολόγιο να συμπληρωθεί από τον γονέα μαζί με το παιδί.

Παρακαλούμε, για όλες τις ερωτήσεις, κυκλώστε το νούμερο που περιγράφει καλύτερα πως αισθανόσασταν την περασμένη εβδομάδα λόγω του άσθματος σας.

Την περασμένη εβδομάδα, ποσο ενοχληθήκατε από:

	Εξαιρετικά ενοχλημένος /η	Πολύ ενοχλημένος /η	Αρκετά ενοχλημένος /η	Κάπως ενοχλημένος /η	Λίγο ενοχλημένος /η	Ελάχιστα ενοχλημένο/ η	Καθόλου ενοχλημένος /η
Ερ8. Βήχα	1	2	3	4	5	6	7
Ερ9. Αναπνευστικό συριγμό	1	2	3	4	5	6	7
Ερ10. Σφίξιμο ή από πόνος στο στήθος	1	2	3	4	5	6	7
Γενικά, πόσο συχνά την τελευταία εβδομάδα νιώσατε.....?							
Ερ11. Δύσπνοια;	1	2	3	4	5	6	7
Ερ12. Κούραση λόγω των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ13. Να έχετε πρόβλημα στο νυχτερινό ύπνο εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ14. Απελπισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ15. Ανήσυχος ή φοβισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ16. Εκνευρισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7

	Εξαιρετικά ενοχλημένος /η	Πολύ ενοχλημένος /η	Αρκετά ενοχλημένος /η	Κάπως ενοχλημένος /η	Λίγο ενοχλημένος /η	Ελάχιστα ενοχλημένο/ η	Καθόλου ενοχλημένος /η
Ερ 17. Διαφορετικός ή απομονωμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Πόσο ενοχλημένος είσασταν την τελευταία εβδομάδα...?							
Ερ 18. Κάνοντας γυμναστική (όπως κολύμπι, τρέξιμο, ανέβασμα /κατέβασμα σκάλες ή ποδήλατο);	1	2	3	4	5	6	7
Ερ 19. Έχοντας επαφή με ζώα (π.χ παίζοντας ή φροντίζοντας κατοικίδια ζώα);	1	2	3	4	5	6	7
Ερ 20. Έχοντας δραστηριότητες με την οικογένεια ή με τους φίλους (π.χ τρέχοντας με τους φίλους στο διαλείμμα στο σχολείο);	1	2	3	4	5	6	7

10a) Physical Activity Level**Q.21. How many times during the week does your child exercise?**

Never/rarely ☐ 1-2 times/week ☐ More than 3 times/week ☐

If YES, what sport does your child participate in/or sports (e.g soccer, basketball, swimming)?

.....

Q.22. For how long does your child play sport?hours/ daytimes/week

If NOT, why?

Does he/she have an asthma attack when he/she plays sport? ☐ Other ☐

Physical Activity Level (Greek)

10b) Φυσική Δραστηριότητα

Ερ21. Πόσες φορές την εβδομάδα αθλείται το παιδί;

Ποτέ/ σπάνια ☐ Μια-δυο φορές/εβδομάδα ☐ Περισσότερο από τρεις φορές/ εβδομάδα ☐

Αν ΝΑΙ, ποιο άθλημα/ή αθλήματα (π.χ ποδόσφαιρο, μπάσκετ, κολύμπι);






Ερ22. Για πόση ώρα αθλείται συνήθως;ώρες/ ημέραφορές/εβδομάδα





Αν ΟΧΙ, για ποιο λόγο;

Παθαίνει κρίση άσθματος κατά την διάρκεια της άσκησης ☐ Άλλο ☐

11 a) Fatty fish pamphlet (Intervention group)

Serving Size of Greek Fatty Fish

FISH PERMITTED (FATTY FISH*)	AMOUNT OF RAW FISH per serve AT LEAST (g)	AMOUNT OF COOKED FISH WITHOUT BONES AT LEAST 150G PER SERVE	ILLUSTRATION OF FATTY FISH FOUND IN GREEK SEAS
Fresh/frozen			
Sardines or Pilchards	12 small or 9 medium-sized sardines (350 g)	150 g	 <p>10-12 small sardines</p>  <p>9 medium sardines</p>
Anchovies	20 pieces (350 g)	150 g	 <p>20 anchovies</p>
Salmon (with bone)	350 g	150 g	
Salmon (Fillet)	250 g	150 g	

FISH PERMITTED (FATTY FISH*)	AMOUNT OF RAW FISH per serve AT LEAST (g)	AMOUNT OF COOKED FISH WITHOUT BONES AT LEAST 150 g PER SERVE	PICTORIAL REPRESENTATION OF GREEK FATTY FISH
Fresh/frozen			
Trout	350 g	150 g	
Chubb Mackerel	350 g	150 g	 1 chubb mackerel
Mackerel	350 g	150 g	 1 medium mackerel
Gilthead Sea Bream	350 g	150 g	

11a) Fatty fish pamphlet (Intervention group)

Fish native to Greek waters NOT TO BE CONSUMED for the purpose of this study:

English Translation of fish found in Greek waters (lean or non-fatty fish)	Greek Name
• White bait	Atherina
• Crayfish	Astako
• Wreckfish	Blaho
• Smooth Hood or Dogfish	Galeos
• Prawns	Garides
• Blue shark	Glaukos
• Common Sole or flounder	Glossa
• Dusky Spinefoot	Germanous
• Bogue	Gopa
• Leather-jackets	Zaketa
• Garfish	Zargana
• Calamari	Kalamari
• Damsel fish	Kalogria
• Streaked Gurnard	Kaponi
• Cuttle Fish	Soupia
• Flathead Mullet	Kefalos
• Red fish or Ocean Perch	Kokkinosparo
• Red Mullet	Koutsomoura
• European Sea Bream or Bass	Lavraki
• Common Pandora	Lithrini
• European Barracuda	Loutsos
• Yellow-tailed Amberjack	Bagiatiko
• Smelt or pikarel	Marida
• Saddled Sea Bream	Melanouri
• Striped Sea Bream	Moumoura
• Cod or Hake	Bakaliaro
• Haddock	Bakaliarakia
• Large-eyed Dentex	Balades
• Striped Red Mullet	Barbounia
• Oysters/mussels	Midia
• Shi drum	Milokopi
• Sharpsnout Sea Bream	Mitaki
• Swordfish	Xifias
• Korean catfish	Pagkasious
• Atlantic Bonito	Palamida
• Monkfish	Peskandritsa






English Translation of fish found in Greek waters (lean or non-fatty fish)	Greek Name
• brown comber	perka
• Blue whiting	prosfiges
• Dusky Grouper	Rofos
• Scad	Savridi
• Skate or Rayfish	Salahi
• Salema	Salpa
• White Sea Bream	Sargo
• Black Sea Bream	Skathari
• Parrot Fish	Skaro
• Red scorpion	Skorpina
• Annular Sea Bream	Sparos
• Golden Grouper	Steira
• Common Dentex	Sinagrida
• White Grouper	Sfirida
• Tuna	Tonos
• Common Sea Bream or Red Porgy	Fagri
• Comber	Hanous
• John Dory	Hristopsaro
• Octopus	Oktapodi
• Fish fingers	Psarokrokates
• Imitation seafood sticks	Apomimisi garidas





REF: <http://www.cretanbeaches.com/en/fauna-and-animal-species/fish-of-crete> , 2009-2016. Accessed on 9.8.16

<http://www.greekdivers.com/mag/el/content/> Accessed on 9.11.16

11b) Fatty fish pamphlet (Greek)

1 ΜΕΡΙΔΑ ΛΙΠΤΑΡΟ ΨΑΡΙ

ΨΑΡΙΑ ΠΟΥ ΕΠΙΤΡΕΠΟΝΤΑΙ (ΛΙΠΤΑΡΑ ΨΑΡΙΑ)*	ΕΛΑΧΙΣΤΗ ΠΟΣΟΤΗΤΑ ΩΜΟ	ΕΛΑΧΙΣΤΗ ΚΑΘΑΡΗ ΠΟΣΟΤΗΤΑ ΧΩΡΙΣ ΚΟΚΑΛΑ ΜΑΓΕΙΡΕΥΤΟ ΨΑΡΙ 150 γρ	ΕΙΚΟΝΑ
Φρέσκα/κατεψυγμένα			
Σαρδέλα	12 μικρές ή 9 μέτριες (350 γρ)	150 γρ	 <p>10-12 Σαρδέλες</p>  <p>9 μέτριες</p>
Γαύρο	20 κομμάτια (350 γρ)	150 γρ	 <p>20 Γαύροι</p>
Σολωμός (φέτα με κόκκαλο)	350 γρ	150 γρ	
Σολωμός (φιλέτο χωρίς κόκκαλο)	250 γρ	150 γρ	


ΨΑΡΙΑ ΠΟΥ ΕΠΙΤΡΕΠΟΝΤΑΙ (ΛΙΠΤΑΡΑ ΨΑΡΙΑ)*	ΕΛΑΧΙΣΤΗ ΠΟΣΟΤΗΤΑ ΩΜΟ	ΜΑΓΕΙΡΕΥΤΟ ΨΑΡΙ (Ελάχιστη καθαρή ποσότητα χωρίς κόκκαλα)	ΕΙΚΟΝΑ
Φρέσκα/κατεψυγμένα			
Πέστροφα	350 γρ	150 γρ	
Κολιός	350 γρ	150 γρ	 1 κολιός
Σκουμπρί (ή Γούνα)	350 γρ	150 γρ	 1 μέτριο σκουμπρί
Τσιπούρα	350 γρ	150 γρ	 1 μέτρια τσιπούρα

11b) Fatty fish pamphlet (Greek)

*Ψάρια/ψαρικά που ΔΕΝ ΕΠΙΤΡΕΠΟΝΤΑΙ	
• Αθερίνα	• Μυλοκόπι
• Αστακό	• Μυτάκι
• Βλάχο	• Ξιφίας
• Γαλέος	• Παγκάσιους
• Γαρίδες	• Παλαμίδα
• Γλάυκος	• Πεσκανδρίτσα
• Γλώσσα	• Πέρκα
• Γερμανούς	• Πρόσφυγες
• Γόπες	• Ροφός
• Ζακέτα	• Σαβρίδια
• Ζαργάνες	• Σαλάχι
• Καλαμάρι	• Σάλπες
• Καλόγρια	• Σαργός
• Καπόνι	• Σκαθάρι
• Κέφαλος	• Σκάρους
• Κοκκινόψαρο	• Σκορπίνα
• Κουτσομούρες	• Σπαρους
• Λαβράκι	• Σουπιές
• Λιθρίνια	• Στήρα
• Λούτσος	• Συναγρίδα
• Μπαγιάτικο	• Σφυρίδα
• Μαρίδα	• Τόνος
• Μελανούρι	• Φαγκρί
• Μουμούρα	• Χάνους
• Μπακαλιάρο	• Χριστόψαρο
• Μπακαλιαράκια	• Χταπόδι
• Μπαλάδες	• Ψαροκροκέτες
• Μπαρμπούνια	• Απομίμηση γαρίδας/καβουρίου
• Μύδια	

12a) Fatty fish/Mediterranean diet poster (Intervention group)

Doctor's orders.....
You have to Eat!




2 serves/per week fatty fish
(sardines, anchovies, trout, salmon, mackerel, chubb mackerel)

(1 serve = 350 g raw/ or 150 g cooked)

Research Team:
Prof C. Tsiopoulos, 00613-94791721, c.tsiopoulos@latrobe.edu.au
Prof Pediatrics, Ch. Katsaris, 210-9322946, katsaris@yahoo.gr
Maria Papamichael, Dietitian, 6947073672, sassipap@hotmail.com

Traditional Greek Mediterranean Diet Pyramid



Daily Beverage Recommendations:
6 Glasses of Water
Wine in moderation

Frequency:
Monthly: MEAT
Weekly: SWEETS, EGGS, POULTRY, FISH
Daily: CHEESE & YOGURT, OLIVE OIL, FRUITS, BEANS, LEGUMES & NUTS, VEGETABLES, BREAD, PASTA, RICE, COUSCOUS, POLENTA, OTHER WHOLE GRAINS & POTATOES

Daily Physical Activity

12b) Fatty fish/Mediterranean diet poster (Greek)



13)a. Weekly fatty fish consumption record(Intervention group)

Instructions: Please record every week the 2 days that your child consumes fatty fish, the type of fatty fish consumed and the amount eaten at each meal.

For example, if your child consumes the first week, say Tuesday 150 g anchovies (15 fish) and on Saturday 200 g trout (weight cooked without head and bones), you will record in 'Week 1', day "Tuesday", 150 g anchovies (15 fish) and for day "Saturday" 200 g trout (see example highlighted in green)

Starting date Consumption of fatty fish/...../.....	Record						
	Consumption of fatty fish						
	2 times /per week at least 150 g cooked fatty fish per meal						
WEEK	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Example		150 g anchovies (15 fish)				200 g trout	
WEEK 1							
WEEK 2							
WEEK 3							
WEEK 4							
WEEK 5							
WEEK 6							
WEEK 7							
WEEK 8							
WEEK 9							
WEEK 10							
WEEK 11							
WEEK 12							
WEEK 13							
WEEK 14							
WEEK 15							
WEEK 16							
WEEK 17							
WEEK 18							
WEEK 19							
WEEK 20							
WEEK 21							
WEEK 22							
WEEK 23							
WEEK 24 Date at 6 months/...../.....							

13b). Weekly fatty fish consumption record (Intervention group) (Greek)

ΟΔΗΓΙΕΣ: Παρακαλώ να καταγράφετε κάθε εβδομάδα τις δύο ημέρες που το παιδί σας καταναλώνει λιπαρό ψάρι και την ποσότητα που καταναλώνει κάθε φορά.

Για παράδειγμα, αν καταναλώνει την πρώτη εβδομάδα, ημέρα Τρίτη 150 γρ. γαύρο (15 ψαράκια) και το Σάββατο 200 γρ. πέστροφα (μαγειρεμένο χωρίς το κεφάλι και κόκκαλα), Θα καταγράψτε στην Εβδομάδα, ημέρα Τρίτη 150 γρ. γαύρο και στη ημέρα Σάββατο 200 γρ. πέστροφα. (βλ. παράδειγμα σε πράσινο φόντο)

ΗΜΕΡΟΜΗΝΙΑ ΕΝΑΡΞΗΣ ΚΑΤΑΝΑΛΩΣΗΣ ΛΙΠΑΡΩΝ ΨΑΡΙΩΝ/...../.....	ΚΑΤΑΓΡΑΦΗ ΚΑΤΑΝΑΛΩΣΗΣ ΛΙΠΑΡΟ ΨΑΡΙ 2 φορές/εβδομάδα τουλάχιστον 150 γ μαγειρεμένο ψάρι ανα γεύμα						
	ΔΕΥΤΕΡΑ	ΤΡΙΤΗ	ΤΕΤΑΡΤΗ	ΠΕΜΠΤΗ	ΠΑΡΑΣΚΕΥΗ	ΣΑΒΒΑΤΟ	ΚΥΡΙΑΚΗ
Παράδειγμα		150 γ γαύρο (15 ψαράκια)				200 γ πέστροφα	
ΕΒΔΟΜΑΔΑ 1							
ΕΒΔΟΜΑΔΑ 2							
ΕΒΔΟΜΑΔΑ 3							
ΕΒΔΟΜΑΔΑ 4							
ΕΒΔΟΜΑΔΑ 5							
ΕΒΔΟΜΑΔΑ 6							
ΕΒΔΟΜΑΔΑ 7							
ΕΒΔΟΜΑΔΑ 8							
ΕΒΔΟΜΑΔΑ 9							
ΕΒΔΟΜΑΔΑ 10							
ΕΒΔΟΜΑΔΑ 11							
ΕΒΔΟΜΑΔΑ 12							
ΕΒΔΟΜΑΔΑ 13							
ΕΒΔΟΜΑΔΑ 14							
ΕΒΔΟΜΑΔΑ 15							
ΕΒΔΟΜΑΔΑ 16							
ΕΒΔΟΜΑΔΑ 17							
ΕΒΔΟΜΑΔΑ 18							
ΕΒΔΟΜΑΔΑ 19							
ΕΒΔΟΜΑΔΑ 20							
ΕΒΔΟΜΑΔΑ 21							
ΕΒΔΟΜΑΔΑ 22							
ΕΒΔΟΜΑΔΑ 23							
ΕΒΔΟΜΑΔΑ 24 Ημερομηνία 6 μήνες/...../.....							

14a) Dietary Habits Questionnaire

Dietary Habits Questionnaire

Q.1. Does your child eat fast food more than once a week (e.g Goody's or Fast-food restaurants)?

YES ☐ NO ☐

Q.2. How many times per week does your child eat breakfast?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Q.3. If your child eats breakfast, does he/she eat one of the following foods and how many times per week?

Breakfast cereal or bread or rusks or toast NO ☐ YES ☐ times/ week.....

Milk products (milk, yogurt, cheese) NO ☐ YES ☐ times/week.....

Cheese-pie or croissant or cookies or cake NO ☐ YES ☐ times/week.....

If NO, what does your child usually eat for breakfast and how many times per week?

.....

Q.4. Do you use olive oil for cooking, frying, or add to pasta and rice?

YES ☐ NO ☐ If NO, what do you use?

FFQ Instructions:

For every food item, indicate the number of times and amount that your child consumes.

For example, if your child drinks **1 cup** of milk **two times per day**, mark “**1**” in the category “**2-3 times/day**”.

If your child eats 2 fruits **once a day**, mark “**X**” in the category ‘**once/day**’.

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Milk (1 Cup)							1	
Fruit (1 medium or ½ Cup)						2		

(1 Cup = 1 Tea Cup (240ml); 1 Tblsp = 1 tablespoon or soup spoon; 1 teas= 1 teaspoon, 1 item=1 piece; 1 tub of yogurt= 200 g)

How many times and serves does your child eat/drink.....?

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Q.5. Milk (1 cup.)								
Q.6. Chocolate milk (1 cup.)								
Q.7. Yogurt (1 tub)								
Q.8. Cheese (White or yellow) (e.g feta or kasseri) (40 g)								
Q.9. Fruit (1 medium)								
Q.10. Fruit Juice (1 cup)								
Q.11. Salads Raw (e.g. Cabbage, rocket, carrots, tomato, lettuce, cucumber) (1/2-1 cup)								
Q.12. Vegetables Boiled (e.g Broccoli, cabbage, cauliflower, collard greens, green beans, marrows, silver beet, spinach, beetroot) (1/2-1 cup)								
Q.13. Stewed Vegetables in sauce (Lady fingers, briam, green beans) (1/2-1 cup.)								
Q.14. Legumes (1 plate =300 g)								
Q.15. Cereals [Breakfast cereals (1/2 cup), bread (1 slice = 30g), rusks (2)]								
Q.16. Pasta (1 cup cooked 140 g)								
Q.17. Rice (1 cup cooked 160 g)								
Q.18. Red Meat e.g [Pork/lamb/ beef / goat/rissoles/bifteki (2 items)] (150 g cooked = restaurant serve)								

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Q.19. White Meat [Chicken/ rabbit/ turkey] (150 g)								
Q.20. Traditional Meals: Pastitsio/ spaghetti bolognese/ / mousaka/ Papoutsakia/ stuffed vegetables with rice (150 g restaurant serve)								
Q.21. Seafood [Calamari, squid, prawns, mussels, octopus] (150 g = restaurant serve)								
Q.22. Fish (Lean) [Pandora, whiting, garfish, smelt, John Dory, white bait, cod, hake, flounder, bogue, swordfish, tuna, red mullet] (150 g = restaurant serve)								
Q.23. Fish (Fatty) [Sardines/anchovies (12 pieces), salmon, trout, mackerel, chubb mackerel, gilthead seabream] (150 g)								
Q.24. Margarine (1 teas)								
Q.25. Nuts (1 handful/ 1/3 cup/ 50g)								
Q.26. Olive Oil (1Tbsp)								
Q.27 Fast Food [Hamburger (1 item), souvlaki with pita (1), pizza (2 pieces) hot dog (1)]								
Q.28. Pies [Cheese-pies, spinach pies] (1 serve = 150 g)								
Q.29. Sweets [Cream cakes (1item) cookies (2), Biscuits (2) cakes (1 piece), croissant (1), ice-cream (1 ball), milk-shake (1 cup), chocolate (60 g)]								
Q.30. Salty snacks [potato chips, twistees, popcorn](1 packet 70 g)								
Q.31. Soft drinks/ Energy drinks (1 can 330 ml)								

Thank you very much!

14b) Dietary Habits Questionnaire (Greek)

Ερωτηματολόγιο Διατροφικών Συνήθειων

Ερ. 1. Το παιδί σας τρώει περισσότερο από μια φορά/εβδομάδα έξω ή φαγητό απ' έξω (π.χ από ψητοπωλείο, μαγαζιά ταχυφαγείας «Goody's»); NAI ☐ OXI ☐

Ερ. 2. Το παιδί σας πόσες φορές/εβδομάδα τρώει πρωινό;

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Ερ. 3. Αν τρώει πρωινό, μήπως τρώει κάποια από τα παρακάτω τροφές και πόσο συχνά;

Δημητριακά ή ψωμί ή φρυγανιές ή τοστ OXI ☐ NAI ☐ φορές/εβδομάδα

Γαλακτομικά (γάλα, γιαούρτι, τυρί) OXI ☐ NAI ☐ φορές/εβδομάδα

Τυρόπιτες ή κρουασάν ή κουλουράκια ή κέικ OXI ☐ NAI ☐ φορές/εβδομάδα

Αν OXI, τι τρώει συνήθως για πρωινό και πόσες φορές/εβδομάδα;

Ερ.4 Χρησιμοποιείται ελαιόλαδο στη μαγειρική, για το τηγάνισμα και όταν φτιάχνετε γεύμα με μακαρόνια ή ρύζι;

NAI ☐ OXI ☐ Αν OXI, τι χρησιμοποιείται;

Ερωτηματολόγιο κατανάλωσης τροφίμων Οδηγίες :

Για κάθε τρόφιμο, σημειώστε η συχνότητα που καταναλώνει το παιδί σας.

Για παράδειγμα, αν πίνει 1 φλιτζάνι γάλα 2 φορές/ημέρα, βάλτε «1» στην κατηγορία 2-3 φορές/ημέρα.

Αν τρώει 2 φρούτα μια φορά την ημέρα, βάλτε «2» στην κατηγορία 1 φορά/ημέρα

Τρόφιμο (μερίδα)	Ποτέ/Σπάνια	1-3 φορές/μήνα	1 φορά/εβδομάδα	2-3 φορές/εβδομάδα	4-6 φορές/εβδομάδα	1 φορά/ημέρα	2-3 φορές/ημέρα	Ισο ή περισσότερο από 4 φορές /ημέρα
Γάλα (1 φλ.)							1	
Φρούτα (1 μέτριο ή ½ φλ.)						2		

(1 φλ.= 1 φλιτζάνι του καπουτσίνο (240ml), 1 κ.σ = 1 κουταλάκι σούπας, 1 κ.γ = 1 κουταλάκι γλυκού, 1 κομ. = 1 κομμάτι,

1 κεσ=1 κεσεδάκι γιαουρτιού (200 γρ))

Πόσες μερίδες και φορές τρώει/ ή πίνει το παιδί σας.....;

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές/ ημέρα	Ισο ή περισσότερο από 4 φορές/ημέρα
Ερ 5. Γάλα (1 φλ.)								
Ερ 6. Σοκολατούχο γάλα (1φλ.)								
Ερ 7. Γιαούρτι (1 κεσ)								
Ερ 8. Τυρί (άσπρο ή κίτρινο) π.χ φέτα ή κασέρι (40 γρ)								
Ερ 9. Φρούτα (1μέτριο)								
Ερ 10. Χυμό φρούτων (1 φλ.)								
Ερ 11. Δημητριακά π.χ [Πρωινού (1/2 φλ), ψωμί (1 φέτα=30 γ), φρυγανιές (2 τεμ.)]								
Ερ 12. Σαλάτα ωμή (π.χ Μαρούλι, λάχανο, ρόκα, καρότα, ντομάτα, αγγούρι) (1/2-1 φλ.)								
Ερ 13. Λαχανικά βραστά (πχ. Μπρόκολο, λάχανο, κουνουπίδι, χόρτα, κολοκύθια, αντίδι, σπανάκι, παντζάρια) (1/2-1 φλ)								
Ερ 14. Λαδερά π.χ (Μπάμιες, μπριάμ, φασολάκια) (1/2-1 φλ.)								
Ερ 15. Όσπρια (1 πιάτο-300 γρ)								
Ερ 16. Μακαρόνια (1 φλ. μαγειρεμένο 140 γρ)								
Ερ 17. Ρύζι (1 φλ. μαγειρεμένο 160γρ)								
Ερ 18. Κρέας κόκκινο [Χοιρινό/αρνί/ μοσχάρι/κατσίκι/ μπιφτέκια (2 τεμ)] (150 γρ μαγειρεμένο= μερίδα εστιατορίου)								
Ερ 19. Κρέας λευκό [Κοτόπουλο/ κουνέλι/ γαλοπούλα] (150γρ)								
Ερ 20. Παστίτσιο/ μουσακά / μακαρόνια με κιμά/ παπουτσάκια/γεμιστά (150 γρ μερίδα εστιατορίου)								

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές /ημέρα	Ισο ή περισσότερο από 4 φορές/ ημέρα
Ερ 21. Θαλασσινά [Καλαμάρι, σουπιές, γαρίδες, μύδια, χταπόδι] (150 γρ =μερίδα εστιατορίου)								
Ερ 22. Ψάρι (2% λιπαρά) π.χ [Σαργός, γόπες, μαρίδα, αθερίνα, φαγκρί, μπακαλιάρος, γλώσσα, λυθρίνια, ξιφίας, λαβράκι μπαρμπούνι, τόνο, κουτσομούρες] (150 γρ = μερίδα εστιατορίου)								
Ερ 23. Ψάρι λιπαρό π.χ [Σαρδέλα/γαύρο (12 κομ), κολιός, σολομός, σκουμπρί, πέστροφα, τσιπούρα] (150 γρ)								
Ερ 24. Μαργαρίνη(1 κ.γ)								
Ερ 25. Ξηρούς Καρπούς (1 χούφτα/1/3 φλ./ 50 γρ)								
Ερ 26. Ελαιόλαδο (1κ.σ)								
Ερ 27. Φαγητό Ταχυφαγείας π.χ [Χάμπουργκερ (1 τεμ), σουβλάκι (1 τεμ), πίτσα (2 κομ.), χοτ ντογ (1 τεμ)]								
Ερ 28. Πίτες π.χ [Τυρόπιτες, κρουασάν, σπανακόπιτες](1 τεμ = 150 γρ)								
Ερ 29. Γλυκά π.χ [Πάστες (1 τεμ.), κουλουράκια (2 τεμ), μπισκότα (2 τεμ), κέικ (1 κομ.), κρουασάν (1 τεμ.), παγωτά (1 μπάλα), μilkσικ (1 φλ), σοκολάτα (60 γρ)]								
Ερ 30. Αλμυρά σνακ π.χ [Πατατάκια, γαριδάκια, πόπκορν] (1 πακέτο 70 γρ)								
Ερ 31. Αναψυκτικά/ Αθλητικά ποτά (1 κουτάκι 330 ml)								

Σας Ευχαριστούμε πολύ!

15a) KIDMED Questionnaire

Participant ID: KIDMED Test

Date: / /

Does your child

Q1. Take a fruit or fruit juice every day? YES ☐ NO ☐Q2. Eat two fruits every day? YES ☐ NO ☐Q3. Eat fresh salad or cooked vegetables regularly once a day? YES ☐ NO ☐Q4. Eat fresh salad or cooked vegetables more than once a day? YES ☐ NO ☐Q5. Eat fish regularly (at least 2-3 times per week)? YES ☐ NO ☐

If YES, how much fish does the participant eat per meal?

60-90 g * ☐ 90-120 g ☐ 120-150 g ☐ More than 150 g ☐

*The amount mentioned is the weight of fish without the bones and head

What type of fish usually eats? e.g. gilthead sea bream, sardines, whiting, salmon

.....

Q6. Go to a fast-food restaurant (hamburger) more than once a week? YES ☐ NO ☐Q7. Eats legumes more than once a week? YES ☐ NO ☐Q8. Eats pasta or rice almost every day (5 or more times per week)? YES ☐ NO ☐Q9. Eats cereals or grains (bread etc.) for breakfast? YES ☐ NO ☐Q10. Eat dairy products for breakfast (yogurt, milk, cheese etc.)? YES ☐ NO ☐Q11. Eat baked goods or pastries (e.g. pies, cookies, croissant) for breakfast? YES ☐ NO ☐Q12. Skips breakfast? YES ☐ NO ☐Q13. Eat nuts regularly (at least 2-3 times per week)? YES ☐ NO ☐Q14. Eat 2 yogurts and/or some cheese (40 g) daily? YES ☐ NO ☐Q15. Eat sweets and candy several times every day? YES ☐ NO ☐Q16. Eat olive oil with meals? YES ☐ NO ☐

15b) KIDMED Questionnaire (Greek)

Όνομα **KIDMED Score (Greek)**

Παρακαλώ απαντήστε με «X» για τα παρακάτω ερωτήσεις:

Το παιδί σαςΕρ 1. Τρώει ένα φρούτο ή πίνει ένα χυμό κάθε μέρα; NAI ☐ OXI ☐Ερ 2. Τρώει δύο φρούτα κάθε μέρα; NAI ☐ OXI ☐Ερ3. Τρώει σαλάτα ή βραστά λαχανικά μια φορά την ημέρα; NAI ☐ OXI ☐Ερ4. Τρώει σαλάτα ή βραστά λαχανικά περισσότερο από μια φορά την ημέρα; NAI ☐ OXI ☐Ερ 5. Τρώει ψάρι συχνά (2-3 φορές/εβδομάδα); NAI ☐ OXI ☐

Αν Ναι, πόσο ψάρι ανα γεύμα;

60-90 γρ μαγειρεμένα * ☐ 90-120 γρ ☐ 120-150 γρ ☐ Περισσότερο από 150 γρ ☐

*Η ποσότητα αναφέρεται στο βάρος του μαγειρεμένου ψάρι χωρίς το κεφάλι και κόκκαλα.

Τι είδος ψάρι τρώει συνήθως π.χ. σαρδέλα, τσιπούρα, μπακαλιάρο
.....

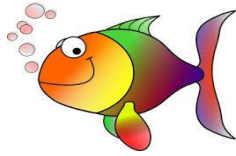
Ερ 6. Τρώει φαγητό απέξω (π.χ. χάμπουργκερ, σουβλάκι) περισσότερο από μια φορά την εβδομάδα;

NAI ☐ OXI ☐Ερ 7. Τρώει όσπρια περισσότερο από μια φορά/ εβδομάδα; NAI ☐ OXI ☐

Ερ 8. Τρώει μακαρόνια ή ρύζι σχεδόν κάθε μέρα (περισσότερο από 5 φορές την εβδομάδα);

NAI ☐ OXI ☐Ερ 9. Τρώει δημητριακά ή ψωμί για πρωινό; NAI ☐ OXI ☐Ερ 10. Τρώει γαλακτομικά (π.χ. γάλα, γιαούρτι, ή τυρί) για πρωινό; NAI ☐ OXI ☐Ερ 11. Τρώει πίτες (π.χ. τυρόπιτες, κουλουράκια) ή κρουασάν για το πρωινό; NAI ☐ OXI ☐Ερ 12. Παραλείπει το πρωινό γεύμα; NAI ☐ OXI ☐Ερ. 13 Τρώει ξηρούς καρπούς συχνά (τουλάχιστον 2-3 φορές/ εβδομάδα); NAI ☐ OXI ☐Ερ. 14 Τρώει 2 γιαούρτια και/ή τυρί (40 γρ) κάθε μέρα; NAI ☐ OXI ☐Ερ 15. Τρώει γλυκά και καραμέλες πολλές φορές κάθε μέρα; NAI ☐ OXI ☐Ερ 16. Χρησιμοποιείται ελαιόλαδο στη μαγειρική στο σπίτι; NAI ☐ OXI ☐

16a) 24-Hour Dietary Recall



METABOLOMIC MEDICINE®
HEALTH CLINICS FOR AUTOIMMUNE AND CHRONIC DISEASES



Βασιλόπουλος
...και του πουλιού το γάλα!



Date:/.....

PATIENT ID:/...../...../.....

What did you eat yesterday?

24 HOUR FOOD RECALL

MEALTIMES	FOOD	AMOUNT	COOKING PREPARATION METHOD
BREAKFAST			
SNACK			
LUNCH			
SNACK			
DINNER			
SNACK			

16b) 24-Hour Dietary Recall (Greek)



METABOLOMIC MEDICINE®
HEALTH CLINICS FOR AUTOIMMUNE AND CHRONIC DISEASES



Βασιλόπουλος
...και του πουλιού το γάλα!



Ημερομηνία :/...../.....

Κωδικός συμμετέχοντα:/...../...../.....

ΑΝΑΚΛΗΣΗ 24 ώρου

Τι φάγατε χθες;

ΓΕΥΜΑΤΑ	ΤΡΟΦΙΜΟ	ΠΟΣΟΤΗΤΑ	ΤΡΟΠΟΣ ΜΑΓΕΙΡΕΜΑΤΟΣ
ΠΡΩΙΝΟ			
ΔΕΚΑΤΙΑΝΟ			
ΜΕΣΗΜΕΡΙΑΝΟ			
ΑΠΟΓΕΥΜΑΤΙΝΟ			
ΒΡΑΔΥΝΟ			
ΠΡΟ-ΥΠΝΟΥ			

16c) Assessment of children's dietary intake during telephone conversations (English)

Prompt questions used by the candidate during telephone interviews to retrieve dietary information on children's, parents' and sibling's eating habits included:

1. Breakfast meal details

-During weekdays does your child consume breakfast?

If yes, what does he/she usually consume for breakfast e.g milk (plain, chocolate, % fat), breakfast cereal, bread, toast, cheese, jam, chocolate spread, cheese-pie, cake, koulouri Thessalonikis, fruit?

Which cereal, whole-meal or sugar-coated?

-Does your child drink milk? How much and how many times per day/ per week? If not, why?

-Does your child drink chocolate milk?

-Do you add cocoa or Ovaltine/Hemo, honey to milk? How much, 1 teas, 1 tbsp.?

-Do you as a parent consume breakfast?

-Do the other children in the family consume breakfast?

-What did your child eat today/or yesterday for breakfast? How much? (portion size, ½ C, 1C, the size of a cappuccino cup)

- How many times per week does your child buy something to eat for breakfast from the local bakery or school canteen? What does he/she usually buy?

-On weekends does your child consume breakfast?

If yes, what does he/she usually eat?

If not, why?

-Does your child eat fruit or drink fruit juice?

If yes, what does he/she usually eat or drink

If not, why?

If yes, how often during the week does he/she eat fruit or drink juice? With added sugar?

2. School recess (on weekdays)

-Does your child eat/or drink something for recess? If yes, what does he/she usually eat?

-Does your child take a sandwich, fruit, yogurt or nuts from home to eat at playtime?

-How do you make sandwiches, with white/wholemeal bread, butter, cheese, ham/turkey, pariser?

-Which cheese do you add? Full fat, low fat? How many slices? If not, why?

-Does your child buy food from the school canteen for recess?

If yes, what does he/she usually buy to eat? Cheese-pie, cake, chocolates, potato chips, croissant, pizza?

-What did your child eat for recess today/ or yesterday? How much? (portion size)

-On weekends, does your child eat a snack before lunch (e.g fruit, cake, cookies, yogurt, croissant or fruit juice)? If yes, how much?

3. Lunch meal

-Does your child eat lunch at school or at home?

If at school, does he/she take a meal prepared at home?

If yes, what does he/she usually eat for the lunch meal?

-Does the meal consist of a source of starch (potato, bread, rice, pasta, legumes), protein (meat, chicken, fish, eggs, legumes, cheese, yogurt) and vegetables/salads, olive oil?

-If your child eats at school, what does the weekly school lunch menu consist of ?

-Does your child eat all of the meal?

-If your child eats lunch at home what does your child usually eat or likes to eat?

-What did your child eat today/ or yesterday for lunch? How much (portion size)?

-Does your child eat bread with meals? If yes, wholemeal/white bread? How many slices?

[In the case where children did not eat a form of starch with meals parents were asked why?]

-Does your child eat vegetables with meals? If yes, which vegetables are consumed, how much (1 cup, 1 fork)? /not consumed? How many times per week? If not, why?

-Do you as a parent consume starch, vegetables with meals?

-Do siblings eat vegetables with meals?

-Does your child eat salads? If yes, with every meal, how much (1 cup, 1 fork) ?

If no, how many times per week and why not?

-Do you as a parent eat salads with meals?

-Do siblings consume salads with meals?

-Does your child eat stewed vegetables? If not, why?

-If yes, which meals are preferred (green beans in tomato sauce, brian, spinach with rice...)

How much does he/she eat? How many times per week?

-Does your child eat bread, cheese or smoked fish with stewed vegetables? If yes how much?

-How many times per week do you cook Traditional Greek Mediterranean dishes (e.g pastitsio, mousaka, stuffed tomatoes with rice/mince, papoutsakia)?

-What size is the portion of your child's meal (small, medium, large)? More than is served in restaurants?

-Does your child eat bread or cheese with meals? How much?

-Does your child eat seafood or fish? If yes, how many times per week. If not, why?

-Which fish does he/she usually eat (whitebait, cod, sardines, sea perch, grilled, fried, fish soup)?

-How often does your child eat red meat (hamburger, spaghetti bolognese, T-bone steak, lamb, goat)?

How much does he/she eat, 1 serve, 2-3 bites?

- Does your child eat vegetables/salads with meat? Why not?
- Does your child eat rice, pasta, bread, or potatoes with meat?
- How often does your child eat white meat (chicken, rabbit, turkey)?
- Does your child eat legumes? If yes, how many times per week and how big is the serve?
- Does he/she eat cheese, bread and smoked fish with legumes? How much? How often?
- Why does your child not eat legumes?
- How do you usually prepare meals, grill, boil, oven-baked?
- How much olive oil do you use in cooking, add to salads?
- Do you use margarine, butter or cream when cooking? Added to pasta or rice?
- Does your child eat cheese (white/yellow) with meals? If yes, how much [1 slice, > 30g), feta cheese with or without added oil?]
- Does your child drink anything with her/his meal? Soft drinks, milk, fruit juice? If yes, how much?

4. Afternoon snack

- Does your child eat something for an afternoon snack or before playing sport?
- If yes, what does he/she usually eat? Cake, cookies, chocolate, milk, yogurt, nuts. fruit, bread, chocolate spread, jam, honey? How much?
- Does your child eat yogurt. If yes, how many times per week and how much?
- If not, does your child eat children's yogurt-type dessert?
- Does he/she add honey, nuts or sugar-coated cereal to yogurt, traditional sugar-boiled fruits (gliko tou koutaliou)?
- Yogurt garlic dip? How many times per week?
- Does your child eat nuts? If yes, how often? If no, why not?
- Is your child allergic to nuts?
- Do you as a parent eat nuts?
- Does your child have a snack after playing sport? For example fruit, sandwich, croissant, chocolate, biscuits, juice? If yes, how much?
- On weekends when your child has more time does he/she eat an afternoon snack? If yes, what does he/she like to eat? How much?

5. Dinner meal

- Does your child consume dinner? If yes, what does he usually eat? How big is the portion (small, medium, large, as served in restaurants?)
- If not, why?

- Do you as a parent eat dinner? Siblings?
- Does your child eat cereal with milk for dinner? If yes, how much?

6. Snack before sleep

- Does your child eat or drink milk before going to sleep?
- If yes, what does your child like to eat/drink?

7. Fast food consumption patterns

- How often does your child eat fast food (e.g Goody's, McDonalds, pizza, souvlaki with pitta, hot dog)?
- Do you order fast food to be delivered home? How often and what do you usually order? If yes, how much does your child eat?
- How often do you go out as a family to eat in restaurants? What does your child usually eat and drink?
- When your child goes out with his/her friends what does he/she usually eat/drink (fried potato chips, pizza, hamburger, waffle, crepes, sweets, milkshake, souvlaki, soft drinks/energy drinks)?
- At parties what does your child usually eat and drink?

8. Sweets consumption

- How many times per day or per week does your child eat sweets (e.g cream cakes, tea cakes, chocolate coated wafers, muffins, croissants, chocolate, lollies, ice cream, waffles, pancakes with chocolate spread and biscuits, biscuits, cookies, ice-cream, Traditional Greek sweets (galaktoboureko, baklava), donuts)?
- How much? When for afternoon snacks or instead of meals?

*When children did not consume a particular meal or food, parents were asked if they consumed that meal/ or food and about siblings eating habits. Also if the child consumed meals alone while parents were working or as a family.

16d) Assessment of children's dietary intake during telephone conversations (Greek)

Prompt questions used by the candidate during telephone interviews to retrieve dietary information on children's, parents' and sibling's eating habits included:

1. Πρωινό

- Καθημερινά όταν πάει το παιδί σας σχολείο, τρώει πρωινό;
 - Αν ναι, τι καταναλώνει συνήθως για πρωινό, π.χ. γάλα (απλό, σοκολάτα, % λίπος), δημητριακά πρωινού, ψωμί, τοστ, τυρί, μαρμελάδα, επάλειψη σοκολάτας, πίτα τυρόπιτα, κέικ, κουλούρι Θεσσαλονίκης;
 - Ποια δημητριακά, ολική άλεσης, με ζάχαρη ή σοκολάτα; (π.χ. Coco Pops, Fitness με σοκολάτα)
 - Το παιδί σας πίνει γάλα; Πόσο και πόσες φορές την ημέρα/ την εβδομάδα; Αν όχι, γιατί;
 - Το παιδί σας πίνει σοκολατούχο γάλα (Μίλκο);
 - Βάζετε κακάο ή Ovaltine / Hemo, μέλι στο γάλα του παιδιού; Πόσο, 1 κ.γ, 1 κ.σ;
 - Εσείς ως γονέας καταναλώνετε πρωινό; Εάν όχι, γιατί;
 - Από τα άλλα παιδιά της οικογένειας, καταναλώνουν πρωινό;
 - Τι έφαγε το παιδί σας σήμερα / ή χθες για πρωινό; Πόσο? (μέγεθος μερίδας, ½ C, 1C, φλιτζάνι του καπουτσίνου).
- Πόσο συχνά την εβδομάδα αγοράζει κάτι από το φούρνο ή κυλικείο να τρώει για το πρωινό του και τί;
- Στο Σαββατοκύριακο το παιδί σας καταναλώνει πρωινό; Εάν ναι, τι τρώει συνήθως;
 - Εάν όχι, γιατί;
 - Το παιδί σας τρώει φρούτα ή πίνει χυμό φρούτων; Εάν ναι, τι τρώει ή πίνει συνήθως;
 - Εάν όχι, γιατί ή πόσες φορές την εβδομάδα τρώει φρούτα/ ή πίνει χυμό;

2. Στο διάλειμμα.

- Το παιδί σας τρώει / ή πίνει κάτι στο διάλειμμα; Εάν ναι, τι τρώει συνήθως;
- Το παιδί σας παίρνει ένα σάντουιτς, φρούτα, γιαούρτι ή ξηρούς καρπούς από το σπίτι για να φάει στο διάλειμμα;
- Πώς φτιάχνετε το σάντουιτς, με άσπρο ψωμί ή ολικής αλέσεως, βούτυρο, τυρί, ζαμπόν / γαλοπούλα, παρίζακι;
- Ποιο τυρί βάζετε στο τοστ του; με πλήρες λίπος, χαμηλά λιπαρά; Πόσες φέτες;
- Εάν το παιδί σας δεν τρώει τυρί, γιατί όχι ;
- Το παιδί σας αγοράσει φαγητό από το κυλικείο;

Εάν ναι, τι αγοράζει συνήθως για να φάει; Τυρόπιτα, κέικ, σοκολάτες, πατατάκια, κρουασάν, πίτσα;

-Το παιδί σας τι έφαγε σήμερα στο διάλειμμα / ή χθες; Πόσο? (μέγεθος μερίδας)

-Στο Σαββατοκύριακο, το παιδί σας τρώει ένα σνακ πριν από το μεσημεριανό γεύμα (π.χ. φρούτα, κέικ, μπισκότα, γιαούρτι, κρουασάν ή χυμό φρούτων); Εάν ναι, τι και πόσο;

3. Το μεσημεριανό γεύμα

-Το παιδί σας τρώει το μεσημεριανό γεύμα στο σχολείο ή στο σπίτι;

Εάν στο σχολείο, παίρνει έτοιμο γεύμα από το σπίτι;

Εάν ναι, τι τρώει συνήθως για το μεσημεριανό γεύμα;

Αν τρώει στο σχολείο, τι περιλαμβάνει το εβδομαδιαίο μενού στο σχολείου;

-Το γεύμα αποτελείται από μια πηγή αμύλου (πατάτα, ψωμί, ρύζι, ζυμαρικά, όσπρια), πρωτεΐνες (κρέας, κοτόπουλο, ψάρι, αυγά, όσπρια, τυρί, γιαούρτι) και λαχανικά / σαλάτες, ελαιόλαδο;

-Το παιδί τρώει όλο το φαγητό του;

-Αν το παιδί σας τρώει το γεύμα στο σπίτι τι τρώει συνήθως το παιδί σας ή του αρέσει να φάει;

-Τι έφαγε το παιδί σας σήμερα / ή χθες για το μεσημεριανό γεύμα; Πόσο (μέγεθος μερίδας);

-Το παιδί σας τρώει ψωμί με τα γεύματα; Εάν ναι, ολικής άλεσης / λευκό ψωμί; Πόσες φέτες;

[Στην περίπτωση που τα παιδιά δεν τρώνε κάποια μορφή αμύλου με γεύματα, οι γονείς ρωτήθηκαν γιατί;]

-Το παιδί σας τρώει λαχανικά με τα γεύματα; Εάν ναι, ποια λαχανικά καταναλώνονται, πόσο (1 φλ.) / ή δεν καταναλώνονται;

-Πόσες φορές την εβδομάδα τρώει λαχανικά;

Εάν όχι, γιατί;

-Εσείς ως γονείς καταναλώνετε άμυλο και λαχανικά με τα γεύματα σας;

-Τα αδέλφια τρώνε λαχανικά με τα γεύματα;

-Το παιδί σας τρώει σαλάτες; Αν ναι, με κάθε γεύμα, πόσο; 1 πιάτο, μια πιρουνιά;

Εάν όχι, πόσες φορές την εβδομάδα και γιατί όχι;

-Εσείς ως γονέας τρώτε σαλάτες με τα γεύματα;

-Τα αδέλφια καταναλώνουν σαλάτες με τα γεύματα;

-Το παιδί σας τρώει λαδερά; Αν ναι, πόσες φορές την εβδομάδα και ποια γεύματα προτιμά (φασολάκια, μπριάμ, μπάμιες, σπανακόρυζο, γεμιστά, αρακάς, ιμάμ μπαλντί). Πόσο τρώει;

Αν όχι γιατί;

Αν ναι, το συνοδεύεται με ψωμί και φέτα τυρί ή καπνιστό ψάρι; Πόσες φέτες ψωμί; Πόσο τυρί (30γρ, 50 γρ, 100 γρ). Πόσο ελαιόλαδο βάζετε στην κατσαρόλα; Πόσο καπνιστό ψάρι τρώει;

-Πόσες φορές την εβδομάδα μαγειρεύετε παραδοσιακά Ελληνικά φαγητά (π.χ παστίτσιο, μουσακά, γεμιστά με κιμά, παπουτσάκια);

Αν ναι, τι του αρέσει να τρώει και πόσο (παιδική μερίδα, ή όπως στο εστιατόριο ή μεγαλύτερη);

Τρώει ψωμί ή τυρί μαζί, πόσο;

-Το παιδί σας τρώει θαλασσινά ή ψάρια; Αν ναι, πόσες φορές την εβδομάδα. Τι τρώει, Ποια ψαριά (γαύρο, μπακαλιάρο, τσιπούρα, καλαμάρι, τηγανιτά, ψητά, σούπα);

Εάν όχι, γιατί;

Πόσο συχνά το παιδί σας τρώει κόκκινο κρέας (χάμπουργκερ, μακαρόνια με κιμά, μπριζόλα, μοσχάρι κοκκινιστό, μπιφτέκια, μπριζόλα);

Τι τρώει; Μοσχάρι, χοιρινό, αρνί, κατσίκι;

Πόσο τρώει, 2-3 μπουκιές, μικρό/ μεγάλη μερίδα;

Πόσο συχνά τρώει άσπρο κρέας (κοτόπουλο, κουνέλι, γαλοπούλα); Πόσο μια μερίδα, λιγότερο;

-Τρώει λαχανικά ή σαλάτες με τα κρέατα; Αν όχι γιατί;

-Τρώει ψωμί, μακαρόνι, ρύζι, ή πατάτες μαζί με το κρέας του;

-Το παιδί σας τρώει όσπρια; Αν όχι γιατί;

-Εάν ναι, πόσες φορές την εβδομάδα και πόσο είναι η μερίδα του (1 μικρό μπολ, ½ κούπα)

-Τρώει ψωμί μαζί με τα όσπρια, ή τυρί (πόσο), ή καπνιστό ψάρι, ή ελιές; Πόσο;

-Πώς μαγειρεύετε τα γεύματα συνήθως, στη σχάρα, κατσαρόλα ή στο φούρνο;

-Πόσο ελαιόλαδο χρησιμοποιείτε για μαγείρεμα ή προσθέστε σε σαλάτες/λαχανικά, μακαρόνι και ρύζι; 1 κούπα, με το μάτι;

-Χρησιμοποιήσετε μαργαρίνη, βούτυρο ή κρέμα στο μαγείρεμα; Στα μακαρόνια, ρύζι;

-Το παιδί σας τρώει τυρί (λευκό/κίτρινο) με τα γεύματα; Εάν ναι, πόσο [1 φέτα, > 30 γρ, με ή χωρίς λάδι;]

-Το παιδί σας πίνει κάτι με το γεύμα του; Αναψυκτικά, γάλα, χυμοί φρούτων, ενεργειακά ποτά; Εάν ναι, πόσο και πόσες φορές την εβδομάδα;

4. Το απογευματινό σνακ

-Το παιδί σας τρώει κάτι κανένα σνακ το απόγευμα ή πριν τη γυμναστική του;

Εάν ναι, τι τρώει συνήθως; κέικ, μπισκότα, σοκοφρέτα, γάλα, γιαούρτι, ξηροί καρποί. φρούτα, ψωμί, μαρμελάδα, μέλι, Μερέντα; Πόσο?

-Το παιδί σας τρώει γιαούρτι. Εάν ναι, πόσες φορές την εβδομάδα και πόσο;

-Προσθέτει μέλι, καρύδια ή δημητριακά με ζάχαρη ή γλυκό του κουταλιού στο γιαούρτι του ή τρώει το παιδικό γιαούρτι επιδόρπιο;

-Τζατζίκι; Πόσες φορές την εβδομάδα;

-Το παιδί σας τρώει ξηρούς καρπούς; Εάν ναι, πόσο συχνά; Εάν όχι, γιατί όχι;

- Είναι το παιδί σας αλλεργικό σε ξηρούς καρπούς;
- Έσείς τρώτε ξηρούς καρπούς;
- Το παιδί σας τρώει κανένα σνακ μετά τη γυμναστική του; Για παράδειγμα φρούτα, σάντουιτς, κρουασάν, σοκολάτα, μπισκότα, χυμό; Εάν ναι, τι και πόσο;
- Στα Σαββατοκύριακα, όταν το παιδί σας έχει περισσότερο χρόνο, τρώει κανένα απογευματινό σνακ; Εάν ναι, τι τρώει; Πόσο;

5. Το βραδινό γεύμα

- Το παιδί σας καταναλώνει δείπνο; Εάν ναι, τι τρώει συνήθως; Πόσο μεγάλη είναι η μερίδα (μικρό, μεσαίο, μεγάλο, όπως σε εστιατόρια;)
- Εάν όχι, γιατί;
- Έσείς τρώτε βραδινό; Τα αδέλφια;
- Το παιδί σας τρώει δημητριακά με γάλα ή σκέτο για δείπνο; Εάν ναι, ποιο δημητριακό και πόσο;

6. Προ-υπνού

- Το παιδί σας τρώει κάτι ή πίνει γάλα πριν πάει για ύπνο;
- Εάν ναι, τι τρώει το παιδί σας;

7. Κατανάλωση φαγητό ταχυφαγείας

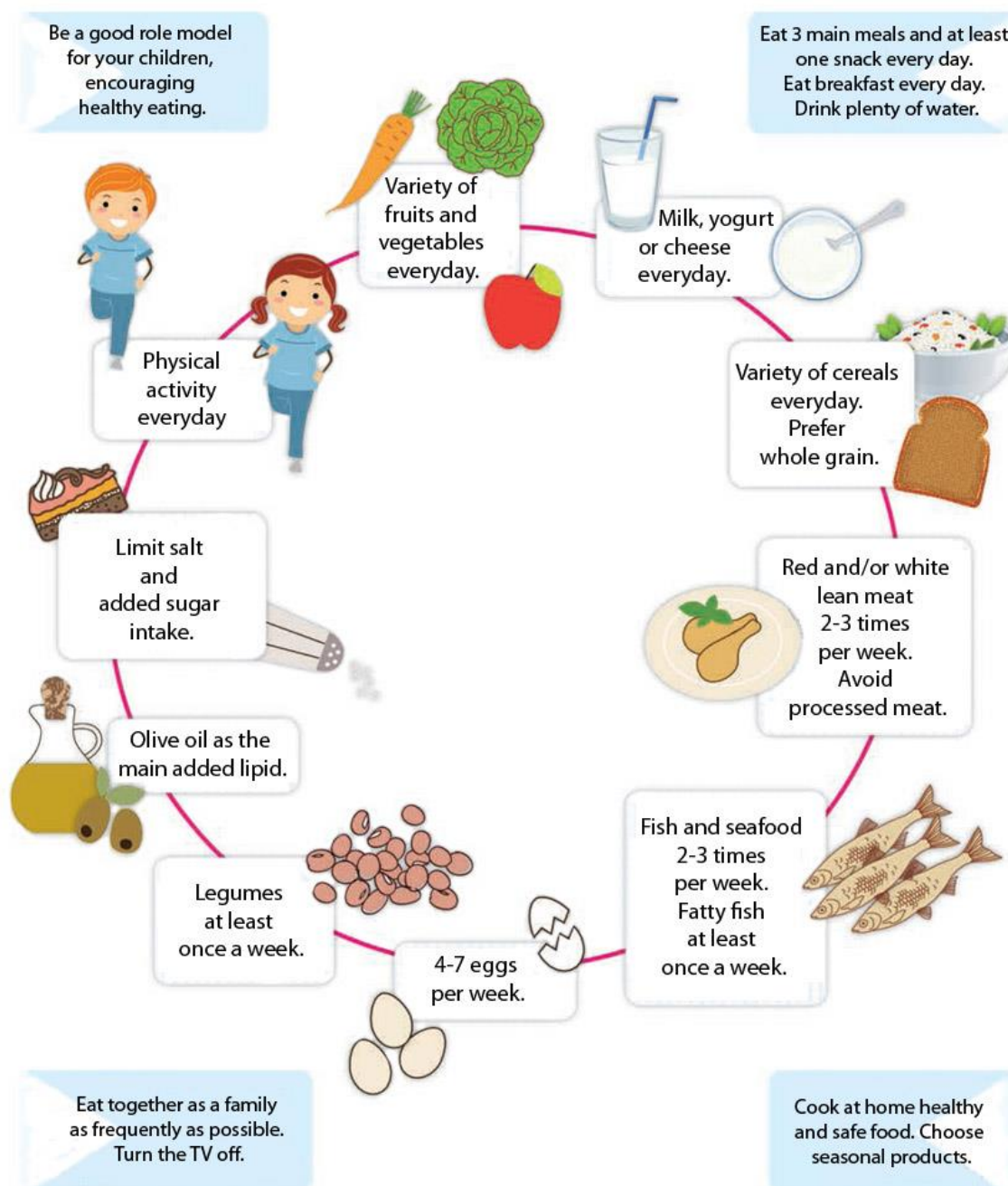
- Πόσο συχνά το παιδί σας τρώει φαγητό ταχυφαγείας (π.χ. Goody's, McDonalds, πίτσα, σουβλάκι με πίττα, hot dog);
- Παραγγείτε γρήγορο φαγητό για το σπίτι; Πόσο συχνά και τι συνήθως παραγγείτε;
- Εάν ναι, πόσο τρώει/ πίνει το παιδί σας;
- Πόσο συχνά βγαίνετε έξω ως οικογένεια για να φάτε στα εστιατόρια; Τι τρώει και πίνει το παιδί σας συνήθως;
- Όταν το παιδί σας βγαίνει με τους φίλους του τι τρώει / πίνει συνήθως (τηγανιτές πατάτες, πίτσα, χάμπουργκερ, βάφλα, κρέπες, γλυκά, παγωτό, σουβλάκι, αναψυκτικά / ενεργειακά ποτά);
- Σε πάρτι τι τρώει και πίνει το παιδί σας συνήθως;

8. Γλυκά

- Πόσες φορές ημερησίως ή εβδομαδιαίως το παιδί σας τρώει γλυκά (π.χ. κέικ κρέμας, κέικ τσαγιού, γκοφρέτες σοκολάτας, muffins, κρουασάν, σοκολάτα, καραμέλες, παγωτά, βάφλες, κρέπες με σοκολάτα και μπισκότα, μπισκότα, παγωτό, παραδοσιακά γλυκά (γαλακτομπούρεκο, μπακλαβά), ντόνατς;
- Πόσο? Πότε, για απογευματινά σνακ ή αντί γεύμα;

End of Study Nutrition Education Material (Intervention /Control group)

17a) Ten Steps to Healthy Eating for Children and Adolescents (PROLEPSIS)



Ref: Institute of Preventive Medicine, Prolepsis 2016. Available from www.diatrofikoiodigoi.gr

17b). Ten Steps to Healthy Eating for Children and Adolescents (PROLEPSIS) (Greek)



Ref: Institute of Preventive Medicine, Prolepsis 2016. Available from www.diatrofikoiodigo.gr

End of Study

18a). Participant Evaluation Questionnaire (Control group)

Participant Evaluation (Control group)

On behalf of the research group we would like to thank you for participating in this study. We would appreciate if you would take a few minutes to complete the study evaluation. Your opinion will help us provide a better service to our patients in future trials.

A. Study evaluation

Please mark with an “X” your response to the following questions:

QA1. The questionnaire format was easy to understand

☐ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA2. Support was provided by the research team at all times

☐ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA3. Have you made any changes in the child’s diet during the intervention as compared to the family’s dietary habits at the start of the intervention?

☐ Yes ☐ No

If yes, what changes have you made to the child’s diet?

.....

QA4. Do you believe that this intervention improved your child’s health and asthma status?

☐ Yes ☐ No ☐ I don’t know

If yes, indicate how?

.....

QA5. Do you believe that this intervention improved your child’s well-being and quality of life (able to engage better in daily activities-sport, play, study etc.)

☐ Yes ☐ No ☐ I don’t know

If yes, indicate how?

.....

B. Future Studies

QB1. Would you be interested in taking part in other dietary interventions in the future?

☐ Yes ☐ No ☐ Maybe

QB2. Do you think that we can improve this intervention in anyway? ☐ Yes ☐ No

If yes, please suggest how?

.....

Thank you!!!!!!!!!!!!

18b). Participant Evaluation Questionnaire (Control group) (Greek)

Αξιολόγηση Ασθενών (Ομάδα Ελέγχου)

Εκ μέρους της ερευνητικής ομάδας θα θέλαμε να σας ευχαριστήσουμε που συμμετείχατε στη μελέτη αυτή. Θα εκτιμούσαμε αν αφιερώστε λίγα λεπτά για την αξιολόγηση της. Η γνώμη σας θα μας βοηθήσει να παρέχουμε καλύτερες υπηρεσίες στους ασθενείς μας σε μελλοντικές μελέτες.

A. Αξιολόγηση της μελέτης

Παρακαλώ, βάλτε «X» στην απάντηση που σας ταιριάζει.

EA1. Η μορφή του ερωτηματολογίου ήταν εύκολα κατανοητή;

☐ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA2. Δόθηκε υποστήριξη από την ερευνητική ομάδα;

☐ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA3. Έχετε κάνει αλλαγές στην διατροφή του παιδιού κατά τη διάρκεια της παρέμβασης σε σύγκριση με τις διατροφικές συνήθειες της οικογένειας πριν την έναρξη της παρέμβασης;

☐ Ναι ☐ Όχι

Εάν ναι, ποιες αλλαγές έχετε κάνει;

.....

EA4. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την κατάσταση υγείας και του άσθματος στο παιδί σας;

☐ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς;

.....

EA5. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την ποιότητα ζωής του παιδιού σας (π.χ συμμετέχει καλύτερα στις καθημερινές δραστηριότητες-άθλημα, παιχνίδι, μελέτη κλπ.);

☐ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς;

.....

B. Μελλοντικές μελέτες

EB1. Θα σας ενδιέφερε να συμμετάσχετε σε άλλες διαιτητικές παρεμβάσεις στο μέλλον;

☐ Ναι ☐ Όχι ☐ Μπορεί

EB2. Πιστεύετε ότι μπορούμε να βελτιώσουμε αυτή την παρέμβαση; ☐ Ναι ☐ Όχι

Εάν ναι, προτείνετε πώς;

.....

Σας Ευχαριστούμε!!!!!!!

End of Study

18c). Participant Evaluation Questionnaire (Intervention group)

Participant Evaluation (Intervention group)

On behalf of the research group we would like to thank you for participating in this study. We would appreciate if you would take a few minutes to complete the study evaluation. Your opinion will help us provide a better service to our patients in future trials.

A. Study evaluation

Please mark with an “X” your response to the following questions:

QA1. The questionnaire format was easy to understand

☐ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA2. Support was provided by the research team at all times

☐ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA3. Have you made any changes in the child’s diet during the intervention (apart from the increase in fish intake) as compared to the family’s dietary habits at the start of the intervention?

☐ Yes ☐ No

If yes, what changes have you made to the child’s diet?

.....

QA4. Do you believe that this intervention improved your child’s health and asthma status?

☐ Yes ☐ No ☐ I don’t know

If yes, indicate how?

.....

QA5. Do you believe that this intervention improved your child’s well-being and quality of life (able to engage better in daily activities-sport, play, study etc.)

☐ Yes ☐ No ☐ I don’t know

If yes, indicate how?

.....

QA6. How was the child’s attitude regarding fish consumption during the 6 month period?

☐ Positive ☐ Negative ☐ Indifferent

QA7. Was the child’s attitude a barrier for regular fish consumption?

☐ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA8. At any time was there a problem in purchasing fatty fish due to availability or cost?

☐ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA9. Was the preparation of fish meals a problem due to lack of time?

☐ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA10. Did you encounter any problems during this intervention? Yes ☐ No ☐

If yes, please indicate

.....

QA11. Do you feel that this dietary intervention was difficult to apply in your daily family life?

☐ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA12. Now that this intervention has ended, do you intend to maintain 2 fish meals per week as part of your family menu?

☐ Yes ☐ No

If no, why not?

.....

QA13. Would you have preferred to give your child an Omega 3 supplement daily as an alternative to feeding your child fatty fish twice weekly?

☐ Yes ☐ No ☐ I don't know

If yes, why?

.....

B. Future Studies

QB1. Would you be interested in taking part in other dietary interventions in the future?

☐ Yes ☐ No ☐ Maybe

QB2. Do you think that we can improve this intervention in anyway? ☐ Yes ☐ No

If yes, please suggest how?

.....

Thank you!!!!!!!!!!

18d). Participant Evaluation Questionnaire (Intervention group) (Greek)

Αξιολόγηση Ασθενών (Ομάδα Παρέμβασης)

Εκ μέρους της ερευνητικής ομάδας θα θέλαμε να σας ευχαριστήσουμε που συμμετείχατε στη μελέτη αυτή. Θα εκτιμούσαμε αν αφιερώστε λίγα λεπτά για την αξιολόγηση της. Η γνώμη σας θα μας βοηθήσει να παρέχουμε καλύτερες υπηρεσίες στους ασθενείς μας σε μελλοντικές μελέτες.

A. Αξιολόγηση της μελέτης

Παρακαλώ, βάλτε «X» στην απάντηση που σας ταιριάζει.

EA1. Η μορφή του ερωτηματολογίου ήταν εύκολα κατανοητή;

☐ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA2. Δόθηκε υποστήριξη από την ερευνητική ομάδα;

☐ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA3. Έχετε κάνει αλλαγές στην διατροφή του παιδιού κατά τη διάρκεια της παρέμβασης σε σύγκριση με τις διατροφικές συνήθειες της οικογένειας πριν την έναρξη της παρέμβασης;

☐ Ναι ☐ Όχι

Εάν ναι, ποιες αλλαγές έχετε κάνει;

.....

EA4. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την κατάσταση υγείας και του άσθματος στο παιδί σας;

☐ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς;

.....

EA5. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την ποιότητα ζωής του παιδιού σας (π.χ συμμετέχει καλύτερα στις καθημερινές δραστηριότητες-άθλημα, παιχνίδι, μελέτη κλπ.);

☐ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς;

.....

EA6. Πώς ήταν η στάση του παιδιού σχετικά με την κατανάλωση ψαριών κατά τη διάρκεια της περιόδου των 6 μηνών;

☐ Θετική ☐ Αρνητική ☐ Αδιάφορη

EA7. Η στάση του παιδιού σας ήταν εμπόδιο για την τακτική κατανάλωση ψαριών;

☐ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

EA8. Υπήρχε ποτέ πρόβλημα στην αγορά λιπαρών ψαριών λόγω διαθεσιμότητας ή κόστους;

☐ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ9. Η προετοιμασία του γεύματος με ψάρι ήταν πρόβλημα λόγω έλλειψης χρόνου;

☐ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ10. Αντιμετωπίσατε κανένα πρόβλημα κατά τη διάρκεια της παρέμβασης; ☐ Ναι ☐ Όχι

Εάν ναι, πώς;

.....

ΕΑ11. Πιστεύετε ότι αυτή η διατροφική παρέμβαση ήταν δύσκολο να εφαρμοστεί στην καθημερινή οικογενειακή ζωή σας;

☐ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ12. Τώρα που έχει ολοκληρωθεί η παρέμβαση, σκοπεύετε να διατηρήσετε 2 γεύματα ψαριών την εβδομάδα ως μέρος του οικογενειακού σας μενού;

☐ Ναι ☐ Όχι

Αν όχι, γιατί;

.....

ΕΑ13. Θα προτιμούσατε να δίνετε στο παιδί σας ένα συμπλήρωμα ωμέγα 3 ημερησίως αντί για 2 γεύματά λιπαρών ψαριών την εβδομάδα;

☐ Ναι ☐ Όχι ☐ Δεν ξέρω

Β. Μελλοντικές μελέτες

ΕΒ1. Θα σας ενδιέφερε να συμμετάσχετε σε άλλες διαιτητικές παρεμβάσεις στο μέλλον;

☐ Ναι ☐ Όχι ☐ Μπορεί

ΕΒ2. Πιστεύετε ότι μπορούμε να βελτιώσουμε αυτή την παρέμβαση;

☐ Ναι ☐ Όχι

Εάν ναι, προτείνετε πώς;

.....

Σας Ευχαριστούμε!!!!!!!

Title: Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: A randomized controlled trial.

Globally, asthma has become one of the most common chronic diseases in children that continue to rise. It causes morbidity and is one of the most common reasons for hospitalization, emergency visits for medical care and absence from school. In Greece, 1 in 10 children suffer with asthma which causes considerable burden to the child, family and society.

According to recent studies adherence to the Mediterranean diet and consumption of fish seem to reduce asthma prevalence and symptoms in children. In comparison, a diet high in saturated fat, salt and sugar was linked to the development of asthma symptoms.

Recently, we conducted a clinical trial titled “The prophylactic potential of a Mediterranean diet enriched with fatty fish on asthmatic children” undertaken by PhD candidate and dietitian Maria Papamichael of La Trobe University, Melbourne under the supervision of Prof. C. Itsiopoulos and in collaboration with Paediatric Pneumologist and Assoc. Prof. Ch. Katsardis and Dr. D.Tsoukalas.

Researchers from Australia and Greece divided 64 children suffering with asthma to two groups. Approximately half of the children were instructed to consume 2 meals of fatty fish weekly (at least 150g cooked filleted fish per meal) as part of the Greek Traditional Mediterranean diet for a period of 6 months and the other half, their usual diet. At the end of the trial, it was observed that there was a significant decrease in lung inflammation by 14 units in the group consuming fatty fish.

These findings suggest that it might be possible to manage asthma symptoms via a healthy diet. The Greek Traditional Mediterranean diet abundant in vegetables/wild greens and fatty fish could be an easy, safe and efficient way to reduce asthma symptoms in children. Fatty fish such as sardines, anchovies, mackerel, chubb mackerel, trout and salmon are rich sources of omega-3 fatty acids with anti-inflammatory properties.

In conclusion, eating two meals of fatty fish per week as part of a healthy diet such as the Mediterranean diet could significantly reduce bronchial inflammation in children with asthma.

Read the findings of the study in the ‘Journal of Human Nutrition & Dietetics’.

Papamichael M.M., Katsardis Ch., Lambert K., Tsoukalas D., Koutsilieris M., Erbas B., Itsiopoulos C. (2018). *Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial.* J Hum Nutr Diet..<https://doi.org/10.1111/jhn.12609>

Contact- Maria Papamichael (PhDc, Dietitian/Researcher La Trobe University)
Mob: 0030-6947073672; e-mail: sassipap@hotmail.com

«Η επίδραση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρά ψάρια σε παιδιά που πάσχουν από άσθμα»

Παγκοσμίως, το άσθμα έχει καταστεί μία από τις πιο συχνές χρόνιες ασθένειες σε παιδιά, και αυξάνεται συνεχώς. Αποτελεί σύνηθες αίτιο καθημερινής νοσηρότητας, εισαγωγής στο νοσοκομείο, επισκέψεων στο ιατρείο και είναι το σημαντικότερο αίτιο απουσιών μαθητών από το σχολείο. Στην Ελλάδα εμφανίζεται σε 1 στα 10 παιδιά και η επιβάρυνση της νόσου στο παιδί, την οικογένεια και την κοινωνία είναι μεγάλη.

Σύμφωνα με μελέτες που έχουν γίνει μέχρι σήμερα η Μεσογειακού τύπου διατροφή και η κατανάλωση ψαριών από παιδιά, φαίνεται να δρουν προφυλακτικά στην εμφάνιση άσθματος αλλά συσχετίζονται και με τη μείωση των ήδη υπαρχόντων συμπτωμάτων. Αντίθετα, μια διατροφή πλούσια σε κορεσμένα λιπαρά, αλάτι και ζάχαρη προκαλεί την ανάπτυξη των συμπτωμάτων άσθματος.

Πρόσφατα, πραγματοποιήθηκε μια ερευνητική μελέτη με τίτλο «Η επίδραση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρά ψάρια σε παιδιά που πάσχουν από άσθμα» από τη Υπ. Διδάκτωρ του Πανεπιστημίου La Trobe της Μελβούρνης και διαιτολόγο Μαρία Παπαμιχαήλ, με υπεύθυνη καθηγήτρια την Δρ. Κ. Ιτσιοπούλου, σε συνεργασία με τον Παιδοπνευμονολόγο κι Επίκουρο Καθηγητή Παιδιατρικής Δρ. Χ Κατσαρδή και τον Δρ. Δ. Τσουκαλά.

Οι ερευνητές από την Αυστραλία και την Ελλάδα χώρισαν 64 παιδιά σε δύο ομάδες και έδωσαν οδηγίες στα μισά περίπου να καταναλώνουν δύο γεύματα από λιπαρά ψάρια (τουλάχιστον 150 γραμμάρια/ γεύμα) κάθε εβδομάδα, στο πλαίσιο της Μεσογειακής διατροφής και για έξι μήνες και τα υπόλοιπα να ακολουθήσουν τη συνήθη διατροφή τους. Στο τέλος της δοκιμής, διαπιστώθηκε ότι η ομάδα που έτρωγε λιπαρά ψάρια είχε μειώσει τη βρογχική φλεγμονή της κατά 14 μονάδες.

Τα άνω ευρήματα αποδεικνύουν ότι είναι δυνατό να διαχειριστούμε τα συμπτώματα άσθματος μέσω της υγιεινής διατροφής. Η Ελληνική Παραδοσιακή Μεσογειακή διατροφή που είναι υψηλή σε φυτικά τρόφιμα και λιπαρά ψάρια, θα μπορούσε να είναι ένας πολύ εύκολος, ασφαλής και αποτελεσματικός τρόπος για τη μείωση των συμπτωμάτων του άσθματος στα παιδιά. Τα λιπαρά ψάρια, όπως σαρδέλα, γαύρος, κολιός, πέστροφα, σκουμπρί και σολομός, έχουν υψηλή περιεκτικότητα σε ωμέγα-3 λιπαρά οξέα, που έχουν αντιφλεγμονώδεις ιδιότητες. Η μελέτη καταλήγει ότι η κατανάλωση λιπαρών ψαριών έστω δύο φορές την εβδομάδα μπορεί να μειώσει σημαντικά την φλεγμονή των πνευμόνων στα παιδιά με άσθμα.

Διαβάστε το εύρημα της μελέτης στο περιοδικό «Journal of Human Nutrition and Dietetics».

Papamichael M.M., Katsardis Ch., Lambert K., Tsoukalas D., Koutsilieris M., Erbas B., Itsiopoulos C. (2018). *Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial.* J Hum Nutr Diet. <https://doi.org/10.1111/jhn.12609>

Επικοινωνία- Μαρία Παπαμιχαήλ (PhDc, Διαιτολόγος/Ερευνήτρια La Trobe University)
Τηλ: 0030-6947073672; e-mail: sassipap@hotmail.com

20a) Letter of study progress sent to collaborators (end of Phase 1 January, 2017) (English)

Athens 9.1.17

To Basilopoulos Supermarkets AE

Dear Sir/Madame,

We would like to inform you that the first phase of our clinical trial titled “The prophylactic potential of Mediterranean dietary pattern enriched with fatty fish in asthmatic children” has been completed.

In the present study 72 children 5-12 years old suffering with asthma were successfully recruited. Follow-up assessments are scheduled to be undertaken after six months after which data analysis will be conducted to determine useful findings that may improve the respiratory health of asthmatic children in general.

Please find attached a copy of questionnaires exhibiting the supermarket’s logo as evidence of your participation in this study. We will update your company regarding publications of the study at international conferences or in scientific journals.

We thank you

On behalf of the research team we wish you

All the very best for a Happy New Year 2017

Yours Sincerely,

Maria Papamichael

Research Team:

Chief Supervisor: Professor C. Itsiopoulos; tel: 0061394793640, [c.itsiopoulos @latrobe.edu.au](mailto:c.itsiopoulos@latrobe.edu.au)

Pneumologist: Dr. Ch. Katsardis; 210-9322946, katsardis@yahoo.gr

Researcher: Maria Papamichael, Dietitian, 6947073672, sassipap@hotmail.com

20b). Letter of study progress sent to collaborators (end of Phase 1 January, 2017) (Greek)

Αθήνα 9.1.17

Προς Βασιλόπουλος ΑΕ

Αξιότιμοι Κύριοι/ες,

Χρονιά Πολλά και Καλή Χρονιά!

Με χαρά σας ενημερώνουμε ότι ολοκληρώθηκε η πρώτη φάση της κλινικής μελέτης με τίτλο «**Η θεραπευτική δράση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρό ψάρι σε παιδιά που πάσχουν από άσθμα**».

Στη μελέτη συμμετείχαν επιτυχώς 72 παιδιά ηλικίας 5- 12 ετών με χρόνια προβλήματα άσθματος. Σε έξι μήνες οπότε και θα επαναληφθούν οι μετρήσεις, θα ολοκληρωθεί η δεύτερη φάση της μελέτης και θα εξαχθούν χρήσιμα συμπεράσματα για την βελτίωση της υγείας των ασθματικών παιδιών εν γένει.

Σας επισυνάπτουμε δείγμα ενός από τα ερωτηματολόγια που χρησιμοποιούνται με το λογότυπο των χορηγών και θα σας ενημερώνουμε για οποιαδήποτε δημοσίευση της μελέτης σε παγκόσμια συνέδρια ή επιστημονικά περιοδικά.

Σας ευχαριστούμε θερμά.

Με εκτίμηση

Μαρία Παπαμιχαήλ

Η Ερευνητική Ομάδα

Η Επιβλέπουσα Καθηγήτρια: Δρ. Κ. Ιτσιόπουλος, 0061394793640, [c.itsiopoulos @latrobe.edu.au](mailto:c.itsiopoulos@latrobe.edu.au)

Ο Παιδοπνευμολόγος: Δρ. Χ. Κατσαρδής, 210-9322946, katsardis@yahoo.gr

Η Ερευνήτρια: Μαρία Παπαμιχαήλ Διαιτολόγος, 6947073672, sassipap@hotmail.com

20c). Letter of study progress sent to collaborators end of Phase 1 January, 2017) (English)

Athens 9.1.17

To Sklavenitis Supermarkets AE

Dear Sir/Madame,

We would like to inform you that the first phase of our clinical trial titled “The prophylactic potential of Mediterranean dietary pattern enriched with fatty fish in asthmatic children” has been completed.

In the present study 72 children 5-12 years old suffering with asthma were successfully recruited. Follow-up assessments are scheduled to be undertaken after six months after which data analysis will be conducted to determine useful findings that may improve the respiratory health of asthmatic children in general.

Please find attached a copy of questionnaires exhibiting the supermarket’s logo as evidence of your participation in this study. We will update your company regarding publications of the study at international conferences or in scientific journals.

We thank you

On behalf of the research team we wish you

All the very best for a Happy New Year 2017

Yours Sincerely,

Maria Papamichael

Research Team:

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Researcher: Maria Papamichael, Dietitian, 6947073672, sassipap@hotmail.com

20d) Letter of study progress sent to collaborators (end of Phase 1 January, 2017) (Greek)

Αθήνα 9.1.17

Προς Σκλαβενίτης ΑΕ

Αξιότιμοι Κύριοι/ες,

Χρόνια Πολλά και Καλή Χρονιά!

Με χαρά σας ενημερώνουμε ότι ολοκληρώθηκε η πρώτη φάση της κλινικής μελέτης με τίτλο «**Η θεραπευτική δράση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρό ψάρι σε παιδιά που πάσχουν από άσθμα**».

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Σας ευχαριστούμε θερμά.

Με εκτίμηση

Μαρία Παπαμιχαήλ

Η Ερευνητική Ομάδα

Η Επιβλέπουσα Καθηγήτρια: Δρ. Κ. Ιτσιόπουλος, 0061394793640, [c.itsiopoulos @latrobe.edu.au](mailto:c.itsiopoulos@latrobe.edu.au)

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Η Ερευνήτρια: Μαρία Παπαμιχαήλ Διαιτολόγος, 6947073672, sassipap@hotmail.com

20e). Letter of study progress sent to collaborators (end of Phase 1 January, 2017) (English)

Athens 9.1.17

To Metabolomics Clinic

Dear Sir/Madame,

We would like to inform you that the first phase of our clinical trial titled “The prophylactic potential of Mediterranean dietary pattern enriched with fatty fish in asthmatic children” has been completed.

In the present study 72 children 5-12 years old suffering with asthma were successfully recruited. Follow-up assessments are scheduled to be undertaken after six months after which data analysis will be conducted to determine useful findings that may improve the respiratory health of asthmatic children in general.

Please find attached a copy of questionnaires exhibiting the supermarket’s logo as evidence of your participation in this study. We will update your company regarding publications of the study at international conferences or in scientific journals.

We thank you

On behalf of the research team we wish you

All the very best for a Happy New Year 2017

Yours Sincerely,

Maria Papamichael

Research team:

Chief Supervisor: Professor C. Itsiopoulos; tel: 0061394793640, c.itsiopoulos@latrobe.edu.au

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Researcher: Maria Papamichael, Dietitian, 6947073672, sassipap@hotmail.com

20f). Letter of study progress sent to collaborators (end of Phase 1 January, 2017) (Greek)

Αθήνα 9.1.17

Προς Μεταβολομική Κλινική

Αξιότιμοι Κύριοι/ες,

Χρονιά Πολλά και Καλή Χρονιά!

Με χαρά σας ενημερώνουμε ότι ολοκληρώθηκε η πρώτη φάση της κλινικής μελέτης με τίτλο **«Η θεραπευτική δράση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρό ψάρι σε παιδιά που πάσχουν από άσθμα»**.

Στη μελέτη συμμετείχαν επιτυχώς 72 παιδιά ηλικίας 5- 12 ετών με χρόνια προβλήματα άσθματος. Σε έξι μήνες οπότε και θα επαναληφθούν οι μετρήσεις, θα ολοκληρωθεί η δεύτερη φάση της μελέτης και θα εξαχθούν χρήσιμα συμπεράσματα για την βελτίωση της υγείας των ασθματικών παιδιών εν γένει.

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Σας ευχαριστούμε θερμά.

Με εκτίμηση

Μαρία Παπαμιχαήλ

Appendix 2B Methods & Materials

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Chapter 5 Methods and Materials

5.1 Study design

Why conduct a randomized controlled trial?

Rationale

Randomised Controlled Trials (RCTs) are considered to be the gold-standard in evaluating healthcare interventions ⁽¹⁾. Unlike observational studies, RCTs are the most robust of all study designs in determining whether a cause-effect relationship exists between treatment and outcome, and for assessing the cost effectiveness of a treatment as well as adverse effects. There are several important features that must be taken into consideration. Firstly, participants must be randomly allocated into the comparison groups (intervention vs control) in order to minimize selection bias. Secondly, participant and clinicians should be unaware of which treatment was given until the end of the study (double-blinding). Also, all intervention groups must be treated identically except for the treatment group. Furthermore, analysis is focused on estimating the size of the difference in outcomes between intervention groups. The advantage of randomization is that it eliminates bias in treatment assignment. It also facilitates masking to the identity of treatments from investigators, participants, assessors and it ensures that the likelihood that any difference in outcome between groups is due to chance. The process of randomization is executed before the study commences, but after subjects have been screened, assessed for eligibility and recruited. There are a number of study designs for RCTs: parallel, cross-over, cluster and factorial. For the purpose of this research study, parallel was chosen because each participant was randomly assigned to a group, and was treated equally, that is, all the participants in the group either received or did not receive an intervention. A limiting factor of RCTs, is that these studies are generally more costly and time consuming compared to other study designs (for example cross-sectional studies). Therefore, costs, duration of study, number of subjects participating in the present trial were taken into consideration ⁽¹⁾.

Study Logo: AUSMED ASTHMA

5.2 Subjects

Why Greek children living in Greece?

Rationale

The prevalence of paediatric asthma, has been documented worldwide. The asthma epidemic continues to increase in all countries even in Mediterranean countries including Greece, albeit at a slower rate ⁽²⁾. Since the dietary intervention entailed adherence to the Mediterranean diet ⁽³⁾, it was easier to use a population that was familiar with the Mediterranean diet prototype than in a non-Mediterranean region such as Australia. It would have been more difficult for Australian children to make drastic changes in dietary habits in a short period of time, thus confounding the results. If the outcome of this clinical trial demonstrates positive effects on asthma outcome in Greek children, then it would be worthwhile to examine whether adoption of a Mediterranean-type diet enriched with fatty fish could have the same benefits in Australian asthmatic children.

Determination of age-group 5-12 years old

Rationale

Participants were selected in the 5-12 years age-range for a number of reasons. Medical studies have reported that asthma onset early in life, during infancy, can be described as ‘transient’. Meaning that, it is often the result of respiratory infections and in most cases dissipates after the age of 3 years old, and that “true” asthma manifests by the age of 6 years ⁽⁴⁾. Furthermore, children aged 5-12 years have developed cognitively so that they are able to understand and follow instructions required in this study ⁽⁵⁾. In addition, pulmonary function tests are indicative in children of at least 5 years of age ⁽⁶⁾. Therefore, it was considered that school children (5-12 years old) were most suitable to participate in this RCT.

Why children with ‘mild’ asthma?

The majority of children diagnosed with asthma suffer from ‘mild asthma’ ⁽⁷⁾. Only 5-10% of the paediatric population are categorized as having severe or chronic asthma ⁽⁸⁾.

5.3 Participant recruitment forms

Information sheet/consent and withdrawal forms

The participant information sheet, consent and withdrawal forms were designed by the candidate according to La Trobe University Human Ethics criteria. Please refer to Appendix 2A, 2a/b, 3a/b and 4a/b for English and Greek translations. The information sheet outlined briefly the study protocol with respect to project aims, intervention and diagnostic tests. It also stated that participation was voluntary, ensured anonymity, confidentiality and the intention of project publication in conferences and scientific journals.

5.4 Study Implementation

Recruitment into this clinical trial commenced after parents/ carers of participants had been informed about study details which included aims, intervention, questionnaires, diagnostic tests involved and signed the consent forms. The first participant was enrolled into the study on 11th November 2016 at 10.30 a.m. and terminated officially on 31st September at 9 p.m., after data from all participants had been collected at the six month follow-up (pulmonary function tests, questionnaires, telephone interviews completed and biochemical test results).

5.5 Procedure

Screening/ Recruitment.

As mentioned in the published study protocol paper, screening and recruitment of participants in this project was conducted by the pneumologist during usual medical consultations according to eligibility criteria (Appendix 2A, 5a/b). In addition, participants were verbally asked by the specialist if they 'liked fish' and would consume fish meals for six months. After written consent, eligible participants were allocated randomly by the physician into one of two arms (intervention versus control groups) with a 1:1 allocation ratio. Children were assessed in the following sequence: anthropometry, spirometry and FeNO analysis. In some participants, when considered necessary to assist asthma diagnosis, the pneumologist performed pulse oximetry. During clinical assessment of participants, parents were used as surrogates to complete the composite questionnaire which included socio-demographics, asthma control, quality of life, physical activity as well as the dietary habits questionnaire. At all times staff at the clinic and the candidate were available to respond to participant's and parents' queries. Completed questionnaires were reviewed by the staff/candidate for any missed questions. The completed forms were placed in the

participant's envelope and collected by the candidate. Allocation concealment was ensured until the participant had been recruited into the trial, which occurred after all assessments had been completed at baseline (namely spirometry, fractional exhaled nitric oxide analysis, anthropometry measurements and questionnaires).

Due to time-constraints, the medical questionnaire, KIDMED questionnaire and 24-hour dietary recalls were conducted by the candidate via telephone interview during the same week of the medical examination. Biochemical testing was undertaken at the Metabolomic clinic located in the city area of Athens within 10 days of enrolment into the study. The same procedure was repeated at the six-month follow-up.

5.6 Anthropometry: Measuring height and weight in children

Physical development is determined by genetic and environmental factors. The change over time in physical development largely reflects changes in the socio-economic level, health and well-being of the population ⁽⁹⁾. In paediatrics, monitoring of a child's physical development is important in assessing the growth, nutritional status and well-being of the child including response to treatment ⁽¹⁰⁾. Regular measurements of children and young people allow for early detection of inadequate growth, ensures appropriate health promotion and support is provided for families. Abnormal growth patterns may signify an underlying medical condition or socio-economic problems requiring further examination and treatment (for example malabsorption, an eating disorder, hypertension, obesity) ⁽¹⁰⁾. Childhood obesity is a growing public health problem worldwide since it has adverse effects on health and is a risk factor for asthma development in childhood ⁽¹¹⁾.

With respect to pulmonary function tests, parameters such as age, gender, height, and ethnicity affect lung volume and function in children ⁽¹²⁾. A study was performed by Alexandraki et al in 414 healthy Greek children to describe the relationship among anthropometric parameters and lung function in Greek children ⁽¹³⁾. A statistically significant correlation was found among FEV₁, FVC, FEF_{25-75%} and PEF and the anthropometric variables: height, age, weight and BMI. Height was found to have the highest correlation with all spirometry variables. In Greece, no criterion has been developed to qualify children's height. In practice, children's height can be classified as normal, short or tall stature based on the U.S Centre for Disease Control and Prevention (CDC) criteria ⁽¹⁴⁾.

According to CDC for children 5-12 years of age:

Height < 5th percentile is considered as “short” stature

5- 95th percentile as “normal” stature

>95th percentile as “tall” stature

Hellenic Paediatric Growth Charts

A number of growth charts are available namely, CDC which is based on the American population and Royal College of Pediatrics and Child Health (RCPCH) for children in the UK that are based on the WHO child growth standards 5-19 years old for healthy breastfed children ⁽¹⁴⁾ ⁽¹⁵⁾. The new revised WHO growth charts (2007) have been developed for international use and are based on a multi-ethnic study of healthy, breast-fed children of non-smoking mothers which was undertaken from 1997-2003 ⁽¹⁶⁾. The sample was selected from 6 countries Brazil, Ghana, India, Norway, Oman, and the USA ⁽⁹⁾. These new growth curves suggest “how children should grow” under ideal conditions ⁽⁹⁾ including breast-feeding exclusively for the first 6 months of life and avoidance of smoking by mothers. By 2010, approximately 125 countries that promote breast-milk as the sole source of nutrition during the first year of the child’s life have adopted use of the WHO growth curves as a tool for monitoring a child’s development. One limitation of the WHO growth curve for children aged (0-5 years) is that the growth curve of breast-fed children varies significantly from that of formula-fed children.

On the other hand, anthropometric variations exist between populations due to genetics and environmental factors, hence population-specific growth charts might provide a more accurate estimation of children’s growth status ⁽¹⁷⁾. With respect to infant feeding practices, in Greece, with more mothers in the work-force, formula-feeding as opposed to breast-feeding is a more popular option ⁽⁹⁸⁾. For this reason it was deemed by the Institute of Child Health and the Hellenic government, that national growth charts are more appropriate for use in this population rather than WHO growth charts which is based on breast-fed infants ⁽¹⁸⁾. The Greek paediatric growth charts have been developed using data collected from formula-fed children during the period of 2000-2001 ⁽¹⁹⁾. In 2016, the charts were updated by modifying the definition for obesity and overweight in children according to the revised WHO criteria (2007) and International Obesity Task Force (IOTF) cut-offs ^(20; 21).

Children's growth patterns are assessed by comparing actual weight and height values with appropriate age and sex-specific growth references ⁽²¹⁾. These growth charts consist of a series of percentile curves illustrating the distribution of anthropometric measurements namely weight and height. In practice, a child's growth and nutritional status is evaluated by plotting the child's measure for height and weight on the appropriate growth chart for age and sex and comparing this value against the cut-points provided in the chart (Figure 1).

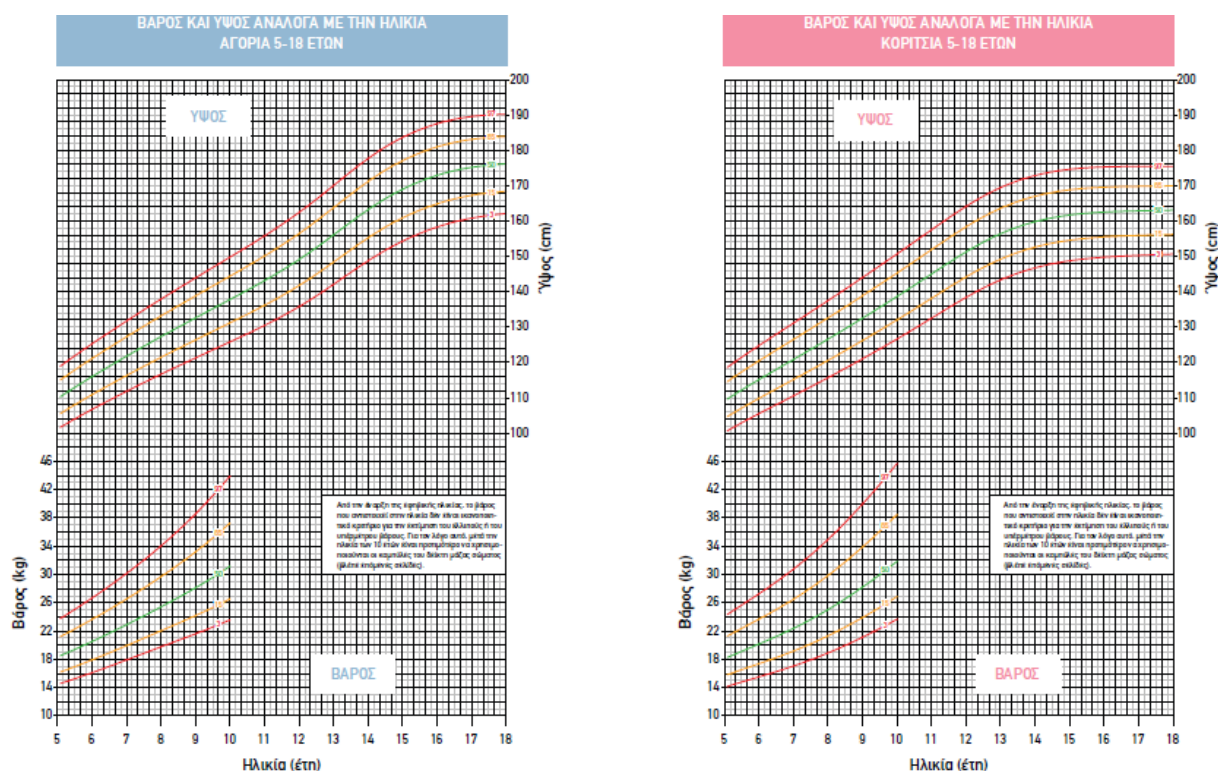


Figure 1 Hellenic Paediatric age-weight and height growth curves for girls and boys aged 5-18 years ⁽¹⁸⁾

Left: Weight-height growth chart for boys aged 5-12 years.

Right: Weight-height growth chart for girls aged 5-12 years.

Figure 1, shows sex-specific paediatric weight and height growth curves for Greek children aged 5-18 years. In both diagrams the coloured curves indicate the 3rd (red), 15th (yellow), 50th (green), 85th (yellow) 97th percentiles (red).

Source: ICH (2017) Hellenic Pediatric Growth Charts. Child Health Book

Available from www.ygeiapediou-ich.gr

On the onset of adolescence, use of the weight-age growth curves underestimate thinness/or underweight and overweight, and BMI growth charts are more appropriate for children older than 10 years ^(18; 22) (Figure 2). Children are classified as normal weight, overweight or obese according to the Hellenic Paediatric Growth Charts ⁽¹⁸⁾ which coincides with IOTF cut-offs ⁽²¹⁾ as follows:

Interpretation of the Hellenic Paediatric Growth Charts.

- BMI plot on or above “30” curve (red) indicates “*obesity*”
- BMI plot that lies on the “25” curve (yellow) or between the “25” and “30” (red) curves indicates “*overweight*”
- BMI plot that lies in between “18.5” (yellow) and “25” (yellow) curves indicates “*normal*” weight.
- BMI plot that lies above “17” and below the “18.5” curve indicates “*slightly thin*”
- BMI plot above “16” and below “17” curves indicate “*thinness*”
- BMI plot below the “16” curve indicates “*severely thin*”

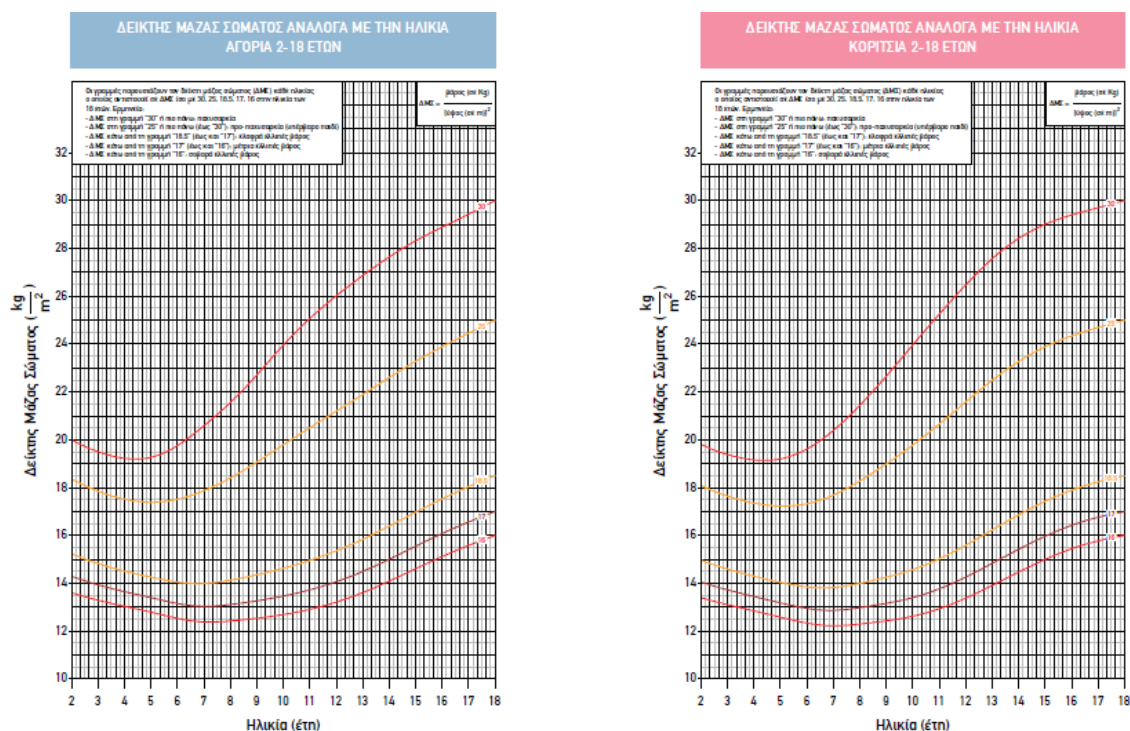


Figure 2. Hellenic Paediatric age versus BMI growth curves for girls and boys ⁽¹⁸⁾

Figure 2 illustrates the BMI growth chart recommended by the Hellenic Ministry of Health for paediatric use to monitor growth and assess overweight, obesity and underweight in healthy children aged 2-18 years ⁽¹⁸⁾. Left illustrates the growth curve for boys and on the right for girls aged 2 -18 years. The colour curves

indicate BMI of 16 (red), 17 (purple), 18.5 (yellow), 25 (yellow) and 30 (red) for adults at 18 years of age. In practice, the BMI of the child is plotted in the diagram according to age and sex. The position of the plot between the colour curves determine whether the child is normal, overweight, obese or thin (slightly thin, thin or severely thin) ⁽¹⁸⁾.

Source: ICH (2017) *Hellenic Paediatric Growth Charts. Child Health Book*

Available from www.ygeiapedidiou-ich.gr

Anthropometry: Measuring height and weight in children

Method

Somatometric measurements were estimated according to WHO protocol ⁽²³⁾. The child's standing height in bare feet was measured to the nearest 0.1 cm using a SECA stadiometer in the standard Frankfort horizontal plane (SECA, Hanover, Germany) ⁽²³⁾. The child was positioned facing the candidate, with feet together, flat on the floor, legs straight, heels, buttocks, back and head against the wall touching the back plate of the measuring instrument and arms loosely by the side ⁽²³⁾ (Figure 3).



Figure 3 Measuring height in children ⁽²³⁾

This figure illustrates the procedure that height was estimated in participants. Children stood in the Frankfort position, with their back aligned to the back plate of the stadiometer.

Source: Centre for Disease Control (CDC) for public domain. Available from https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/measuring_children.html

Children's weight was measured to the nearest 0.1 kg on calibrated electronic scales (SECA weighing scales, Hanover, Germany), without shoes and heavy clothing (e.g coat) (Figure 4). Children were instructed to stand with both feet in the centre of the scale, legs slightly apart and not to move until a reading appeared on the console. Three measurements were taken and the mean value was recorded to the nearest decimal and used for analytical purposes ⁽²³⁾.



Figure 4. Measuring weight in children ⁽²³⁾

Figure 4 depicts measuring weight in participants. Scales were set to 'zero'. Shoes were removed from children and feet positioned in the centre of the scale.

Source: *Centre for Disease Control (CDC) for public domain. Available from*
https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/measuring_children.html

Body Mass Index (BMI)

Body Mass Index was calculated as kg/m^2 for each participant ⁽²³⁾ and recorded by the candidate and spirometry technician. Then the BMI value was plotted on the appropriate BMI growth charts for children aged 2-18 years according to age and sex (Figure 5). Children were classified as normal weight, overweight or obese according to the Hellenic Paediatric Growth Charts which is comparable to the IOTF cut-offs for children 2-18 years ⁽¹⁸⁾.

Application:

For example, a male participant (ID: 70 081008/M/I) of 8 years old had a BMI of 19.71 kg/m^2 . Plotting 19.71 kg/m^2 at age 8 years on the BMI growth curve for boys 2-18 years shows a point that lies within the “ 25 kg/m^2 ” (yellow) and “ 30 kg/m^2 ” (red) curves. According to IOTF criteria ⁽²¹⁾, this area indicates that the child is overweight (Figure 5).

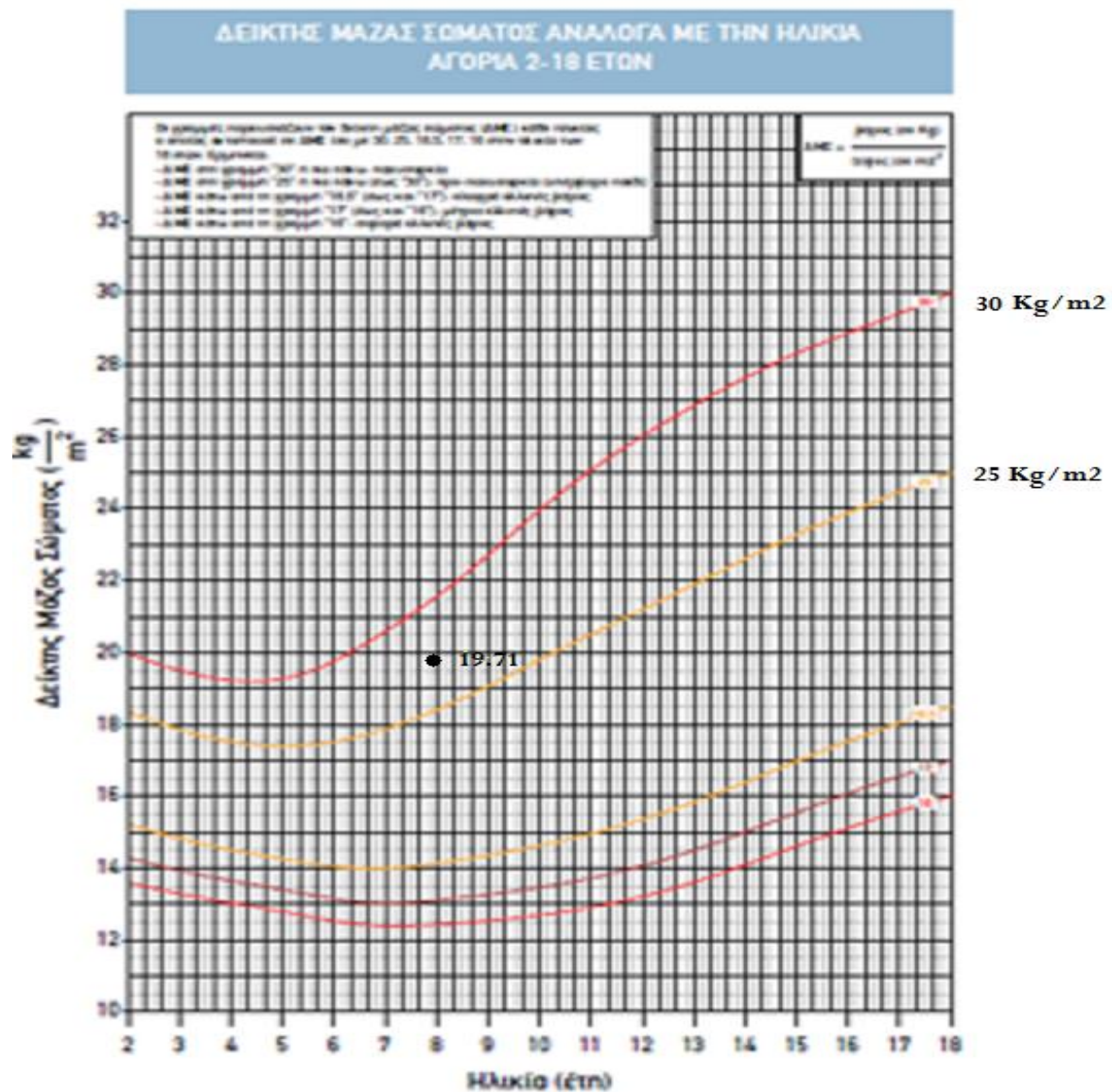


Figure 5. Evaluating weight status in boys using BMI age-sex specific growth charts

This figure illustrates that an 8-year old boy with BMI of 19.71 kg/m^2 is overweight according to the Hellenic Paediatric Growth Charts. The BMI value of 19.71 indicated by the black dot on the diagram lies in the area between BMI of 25 kg/m^2 percentile (yellow curve) and 30 kg/m^2 (red curve).

5.7 Performing Spirometry in children

Rationale

Evaluation of lung function is important in diagnosing, monitoring asthma symptoms and evaluating the effectiveness of asthma therapy on pulmonary function ⁽²⁴⁾. According to GINA guidelines and the European Respiratory Society (ERS), spirometry is the gold standard of pulmonary function tests in children over 6 years ⁽²⁴⁾. It is a non-invasive diagnostic tool assessing the mechanical properties of the pulmonary system ⁽²⁴⁾. A number of conditions affect spirometry performance. The desirable range of temperature in the laboratory should be within 17-40⁰ Celsius. Patients should avoid heavy exercise within 30 min, large meals within 2 hours and for adolescence, alcohol consumption within 4 hours and smoking within 1 hour of testing. Patients are recommended to abstain from short-acting bronchodilator use within 4 hours and long-acting within 12 hours of the test. Before testing, recording of patient's age, sex, ethnicity (e.g Caucasian), height, weight, BMI, and smoking status is necessary since these parameters influence the expected lung volume and ultimately the results ^(6; 12). Lung volume increases as the individual grows from birth to 18-20 years in females and 20-24 years of age in males. The decline in parameters, FEV₁ and FVC is related to age, height and race ⁽¹²⁾. Children have a higher elastic recoil than adults with faster emptying of the lungs, which means that a low FEV₁ does not necessarily indicate early lung disease ⁽¹²⁾. Normal pulmonary function in children ≥ 6 years old is indicative by FEV₁ $\geq 80\%$ predicted ⁽²⁵⁾. Clinical significance is considered to be an increase in FEV₁ $\geq 12\%$ after bronchodilator administration ^(12; 24).

Method

At both time-points, after anthropometry measurements were undertaken in participants by the candidate, spirometry testing was performed by a trained technician according to ERS/American Thoracic Society (ATS) protocol ⁽²⁵⁾. Pulmonary function was measured using a portable spirometer MIR SPIROBANK II (MIR Spirobank II, Medical International Research (MIR) Inc USA) (Figure 6).



Figure 6. MIR Spirobank II

Source: MIR Spirobank II Manual.

Available from <https://www.spirometry.com/ENG/Products/spirobank2.asp>

According to the ERS, SPIROBANK is a validated diagnostic tool for measuring lung function in children ⁽²⁵⁾. Participants were asked to abstain from taking bronchodilators within 4 hours (or 8 hours for long-acting bronchodilators) of the spirometry test. Personal details such as the participant's name, date of birth, sex, ethnicity (Caucasian), smoking/no smoking, weight, height, BMI were recorded in the computer program. Spirometry was conducted twice, pre and post-medication in order to compare ventilatory dynamics. The technician demonstrated how to exhale into the mouthpiece of the spirometry device. Spirometry was conducted in the standing position. A nose clip was placed on the participant's nose in order to prevent nasal inhalation. A new mouthpiece was replaced for each participant. The mouthpiece was inserted into the participant's mouth, lips were sealed around the mouth-piece and a big breathe was inhaled to total lung capacity (TLC) and immediately air was exhaled as hard and as fast as possible without pause, as if blowing out candles on a birthday cake and then a big breathe was inhaled again back to TLC. In order to ensure an acceptable spirogram, the child must not cough, or take an extra breath during the manoeuvre. Exhalation must last for at least 3 seconds in children younger than 10 years and at least 6 seconds in children older than 10 years ⁽¹²⁾. This process was repeated three times and the best value was recorded. The participant was then given 4 inhalations of a bronchodilator, Aerolin (125 mg) and instructed to wait 10-15 minutes before repeating the process. The output of the participant's spirometry readings was printed and forwarded to the physician and the candidate (Figure 7).

ASS. PROF. OF PED. PULMONOLOGY

PAFOU 1, MAROUSI - 210 93.22.946

Visit date 28/11/2016

Patient code 1507	Age	6
Surname ΤΣΕΚΟΥΠΑ	Gender	Female
Name ΕΛΕΝΗ	Height, cm	120
Date of birth 14/7/2010	Weight, kg	21
Ethnic group Caucasian	BMI	14,58
Smoke No smoker	Pack-Year	
Patient group		

Interpretation



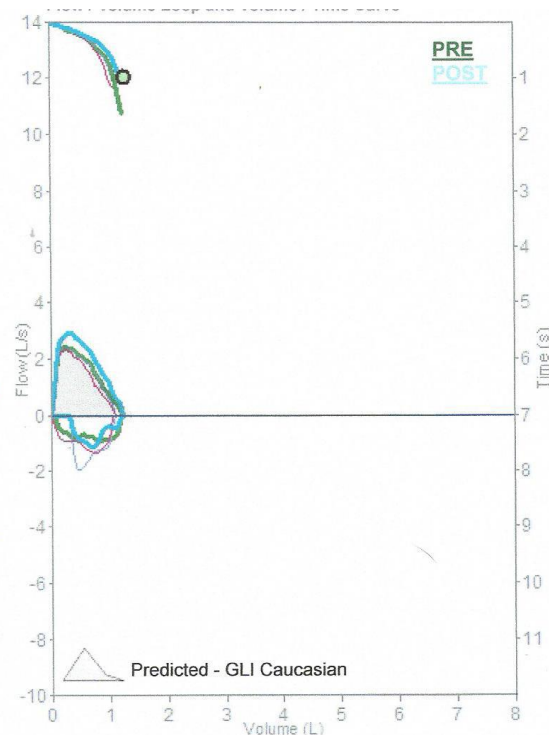
Normal Spirometry

No Significant Bronchodil.

WARNING: CHG FEF2575 = 27%

Best values from all loops

Parameters	LLN	ULN	PRE	%Pred	Z-score	POST	%Chg
FVC L	1,14	1,74	1,24	86	-1,09	1,23	-1
FEV1 L	1,03	1,57	1,14	87	-1,01	1,20	5
FEV1% %	53,7	128,5	91,90	101	0,04	97,60	6
PEF L/s	0,76	5,17	2,49	84		2,94	18



PRE Trial date 28/11/2016 6:05:16 μμ

Parameters	LLN	ULN	Pred	PRE # 1	%Pred	Z-score	PRE # 2	PRE # 3	POST#1	%Pred	%Chg
FVC L	1,14	1,74	1,44	1,24	86	-1,09	1,11	1,09	1,23	85	-1
FEV1 L	1,03	1,57	1,30	1,14	87	-1,01	1,11	1,05	1,20	92	5
FEV1/VC %	53,7	128,5	91,1								
FEV1/FVC %	53,7	128,5	91,1	91,9	101	0,04	100,0	96,3	97,6	107	6
PEF L/s	0,76	5,17	2,96	2,49	84		2,47	2,35	2,94	99	18
FEF2575 L/s	1,09	2,42	1,75	1,72	98	-0,07	1,95	1,66	2,18	125	27
ELA Years			6								
FET s			6,00	1,64	27		0,94	1,18	1,04	17	-37
EVOL mL											
FIVC L	1,14	1,74	1,44	1,16	81	-1,54	0,73	1,07	0,89	62	-23

BTPS 1,12 19 °C 66,2 °F

Conclusion / Medical report

Quality Report

D

Repeatable FVC, Repeatable
FEV1, Repeatable PEF

Signature

Instrument used
Spirobank II S/N 004488

Figure 7. Output of spirometry testing at baseline for a female participant aged 6 years old (ID: 25/140710/F/I) taken on 28.11.2016 at 6 p.m.

This output shows normal pulmonary function since FEV₁, FVC, PEF and FEF_{25-75%} are greater than 80% predicted⁽¹²⁾ and the ratio of FEV₁/FVC greater than 80%⁽¹²⁾.

5.8 Fractional exhaled nitric oxide testing

Rationale

Airway inflammation is a hallmark of asthma ⁽²⁶⁾. Given that airway inflammation is not detected in spirometry tests, FeNO testing provides additional information on the risk of future exacerbation, concordance and effectiveness of pharmacotherapy along with a decline in lung function ⁽²⁶⁾. It is not uncommon for patients with asthma to have normal spirometry measurements ($\geq 80\%$ predicted) with underlying bronchial inflammation ⁽²⁷⁾. According to the National Institute for Clinical Excellence (NICE) guidelines, the Bedfont “NO breathe” FeNO monitor (Bedfont Scientific Ltd, UK) ⁽²⁸⁾ is an easy, non-invasive handheld device that measures FeNO in the breath of patients ⁽²⁹⁾ (Figure 8). This monitor has been designed for use in primary care to measure FeNO in order to diagnose asthma and manage symptoms in paediatric patients as well as in adults. Indications of no bronchial inflammation are considered to be FeNO values $< 20\text{ppb}$ in children ⁽³⁰⁾.



Figure 8. ‘NO breathe’ portable FeNO breathe analyser ⁽²⁸⁾

Source: Bedfont Scientific Limited. Available from <https://www.bedfont.com/nobreath>

Method

At both time-points NO was measured by trained personnel using a portable FeNO analyser “NO Breathe” ⁽²⁸⁾ which according to NICE guidelines is an accredited diagnostic device for measuring FeNO produced during airway inflammation ⁽²⁹⁾. On the day of FeNO testing participants were informed to abstain from eating and drinking one hour before the test. FeNO testing was conducted according to ATS/ERS protocol ⁽³¹⁾.

Participants performed the test in an upright position without nose-clips (Figure 9). The mouthpiece was inserted into the participant’s mouth, lips were sealed around the mouth-piece

and a big breathe was inhaled over 2 to 3 seconds to total lung capacity and then exhaled immediately as hard as possible into the meter at a constant flow rate of 50 ml/sec and mouth pressure of 15 cm H₂O for a period of 10 seconds which allowed a reasonable plateau to be achieved (Figure 9). Three measurements (in ppb) were taken at 30 second intervals that agreed within 10% and the final FeNO which is the mean value was recorded ⁽³¹⁾. Inflammation in children is considered to be a reading ≥ 20 ppb ^(30; 32). The higher the FeNO reading, the greater the level of inflammation in the airways.

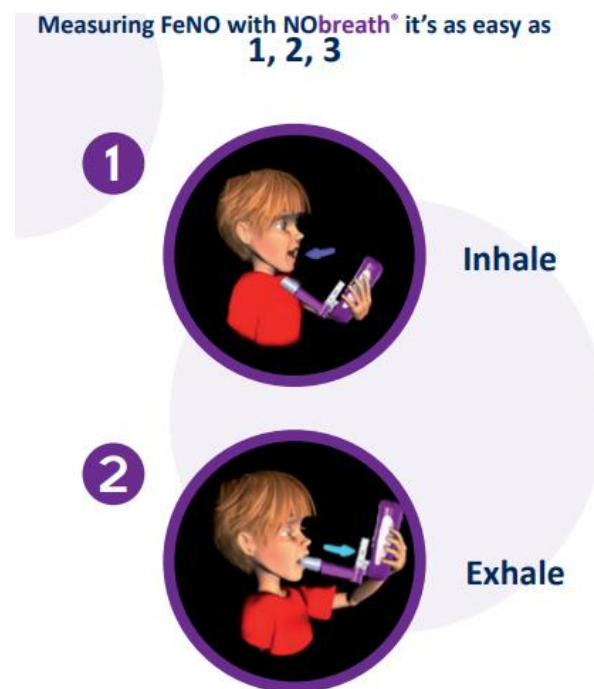


Figure 9. Performing FeNO analysis using Bedfont ‘NO breath’ analyzer is as easy as 1, 2, 3 ⁽²⁸⁾

Figure 9 illustrates a participant performing FeNO analysis with “NO Breathe” Analyzer

Source: Bedfont Scientific Limited. Available from <https://www.nobreathfeno.com/>

5.9 Pulse Oximetry

Rationale

In primary care of paediatric asthma patients, pulse oximetry offers a simple, quick, non-invasive method for measuring blood oxygen levels. It is helpful in monitoring and assessing hypoxia associated with asthma along with the severity of an acute exacerbation or wheezing in children ^(33; 34). In healthy children arterial oxygen saturation (SpO₂) should range between 95 to 100% ⁽³³⁾. Normal heart rate in children aged (6-12 years old) is defined from 75-118 beats/minute ⁽³⁵⁾.

Method

At both time-points, when the pneumologist suspected that the participant was in danger of hypoxia, pulse oximetry was performed as follows:

The participant's index finger was placed into the chamber of the device and within 5-10 seconds a reading of the blood oxygen level (SpO₂%) and pulse rate appeared on the screen. (Figure 10)



Figure 10. Performing pulse oximetry in paediatric patients

Figure 10 shows a participant (ID 25/140710/F/I) performing pulse oximetry. The screen shows a reading of '97%' for blood oxygen indicating normal blood oxygen level. A pulse rate of '81' beats per minute is normal for children within the age range of 6-12 years old ⁽³⁵⁾.

Questionnaires

5.10 Socio-demographics Questionnaire

Rationale:

An important part of research studies are socio-demographics since such information gives a description of the population under investigation as well as identifies risk factors that promote asthma onset ⁽¹¹⁾ which could modify the outcome of this research project.

The following characteristics were retrieved from the literature and assessed in the socio-demographic questionnaire (Table 1).

Table 1. Characteristics assessed in the socio-demographic questionnaire

Socio-demographic details	
Respondent (parent)	Child
Relationship with child (mother/father)	Birth order
Race (maternal/paternal)	Date of birth
Ethnicity (maternal/paternal)	Gender
Marital status	Weight
Employment (maternal/paternal)	Height
Education level (maternal/paternal)	Type of school attended
Monthly income	
Family size	

Measuring Socio-Economic Status

Rationale

In epidemiological studies socioeconomic status (SES) has been measured by a combination of variables including occupation, education, income, wealth and by area of residence or post-code ⁽³⁶⁾. Income and wealth are commonly used as proxy measures for SES but the only disadvantage with these two variables are that many respondents are reluctant to reveal such information, or tend to give false information (reporting bias) ⁽³⁷⁾. In contrast, education level is a good proxy measure for SES for individuals over 25 years, however it does not indicate income or wealth. An advantage is that respondents are willing to answer such questions honestly. Educational attainment is usually measured by highest degree earned (secondary school, college, university) or years of education (1-30 years). Recently, researchers have become interested in using

residential area or post code to measure SES ⁽³⁷⁾. This concept is based on the fact that people that are less economically fortunate (poor) will live in areas dominated by people of the same class and that the wealthier will live with the rich. Also those being wealthier will have access to better education and professions. Therefore, knowing the area where one resides is a good indicator of status and SES ⁽³⁷⁾. As afore-mentioned, the advantages of using education level, area of residence or postcode as indicators of SES are that respondents are more likely to provide these details.

Methods

The socio-demographic questionnaire was a concise, simple questionnaire consisting of 17 questions evaluating parents' details that included SES, educational level, dwelling area, employment status, marital status, ethnicity and race as well as information pertaining to the child such as age, birth order, family size, date of birth, sex, weight, age, and type of school attended (Appendix 2A, 6a/b).

5.11 Medical history questionnaire

Rationale

Part of patient care is the monitoring of asthma control and progress which includes medication use, frequency and severity of asthma symptoms, evaluation of lung function via spirometry, compliance to asthma therapy and efficacy of the prescribed medication as indicated by the absence or presence of minimal symptoms and reduced hospitalization for exacerbations ⁽⁴⁾. It is well-established that allergic rhinitis is a high risk factor for asthma development in children and is associated with poor asthma control, increased risk of hospitalization/emergency visits, reduced quality of life and higher healthcare costs ⁽³⁸⁾. Therefore, monitoring and management of both diseases pharmacologically is crucial in improving quality of life and in decreasing the burden of both conditions ^(38; 39).

For the purpose of this study a concise 18-item medical history questionnaire was designed to collect parents' information regarding health and lifestyle factors that are known risk factors for asthma development in children such as parents' smoking habits, and allergy ⁽¹¹⁾. Also included were questions pertaining to the participant along with pregnancy details namely gestation

period, caesarean delivery, low-birth weight, breast milk or formula feeding, age of asthma onset, associated allergies (rhinitis, eczema and food allergy), medication use over the past month and vitamin intake (Appendix 2A, 7a/b). In order to aid recall, common medications used in the treatment of asthma (such as Short Acting Beta 2 Agonists (SABA), Long Acting Beta 2 Agonists (LABA), Inhaled Corticosteroids (ICS), Anti-leukotrienes) and rhinitis (including corticosteroids and anti-histamine therapy) were indicated by trade-names and represented pictorially.

Methods

At both time-points the 18-item medical history questionnaire was administered by the candidate during telephone interviews. The questionnaire required approximately 15 minutes. In the case where care-takers or grandparents were caring for children, the candidate arranged another time to perform the interview when at least one parent was available at home. At the six month assessment, since parents' history and smoking habits had not changed from baseline, only questions regarding children's allergy, medication and vitamin use (Q15-Q18) were reiterated from the baseline medical questionnaire

5.12 Assessment of asthma control

Rationale:

Population studies have shown that despite the availability of effective drug therapy, in Europe only one in 20 children have adequate asthma control according to GINA guidelines ⁽⁴⁰⁾. There is evidence of underuse of inhaled corticosteroids in asthmatic children and over-use of rescue medication (short-acting β_2 agonists). Both parents and physicians tend to overestimate asthma control ⁽⁴⁰⁾. Hence, a simple, quick method for assessing asthma control in clinical practice is needed.

The evaluation of asthma control in children is an important part of patient care in managing asthma symptoms, preventing future exacerbations and in evaluating effectiveness of asthma therapy in children ⁽⁴¹⁾. Based on GINA recommendations, there are two short validated questionnaires namely the child Asthma Control Test (cACT) ⁽⁴²⁾ and the Asthma Control Questionnaire (ACQ) ⁽⁴³⁾ that have been developed to measure asthma control in paediatric patients ⁽⁴¹⁾. In both questionnaires, asthma control is assessed by evaluating the prevalence of day and night asthma symptoms, activity limitations and use of reliever medication. These tools provide scores and cut-points to distinguish between different levels of symptom control. When

assessing asthma control in children under 12 years old, it is important that parents are included in the retrieval of information from children, because of children's short memory recall ⁽⁴¹⁾.

The Asthma Control Questionnaire (ACQ) is one of the most popular questionnaires used worldwide to evaluate asthma control in patients both children and adults ^(43; 44). It is able to distinguish patients with good asthma control from those with suboptimal control. This questionnaire is applicable for children aged 6-16 years and estimates adequacy of control in patients, detects clinically important changes in lung function and is sensitive to intra-patient change over time ⁽⁴⁵⁾. Therefore, in practice and in clinical trials the ACQ is a valuable and accurate tool for identifying the degree of asthma control experienced by the patient and it can distinguish small changes that occur either spontaneously or as a result of interventions ⁽⁴⁵⁾.

The ACQ is a validated, short and easy self-administered questionnaire for children as young as 6 years. The questionnaire consists of 7 items that assess the presence of symptoms (wheeze, tightness in the chest, dyspnoea, cough), need for daily medication, limitations in daily activities (for example school absenteeism) and pulmonary function by % FEV₁ predicted, during the past 7 days ⁽⁴⁴⁾ (Appendix 2A, 8a/b). Responses for the first 6 questions are based on a 7-point scale (0 = totally controlled to 6 = extremely poorly controlled). The 7th question, regarding spirometry measurement FEV₁, is completed by the clinic staff and a score ranging from 0-6 is assigned based on the value of FEV₁. For example, a value of 92% for FEV₁ is given a score of 0, whereas a value of 65%, a score of 4. The final ACQ score is the mean of scores for the 7 questions. An ACQ score ≤ 0.75 indicates 'well-controlled' asthma, 1-1.5 'not well controlled' and ACQ score ≥ 1.5 as 'un-controlled asthma'. The minimal clinically significant change in score which physicians consider to be beneficial, in the absence of symptoms is 0.5. In clinical practice, an ACQ score ≤ 0.75 , signifies that there is an 85% chance that the patient's asthma is 'well-controlled'. However, in clinical trials, a patient's score of ≥ 1.50 means that there is an 88% chance that the patient's asthma is 'not well-controlled' ⁽⁴⁶⁾. The original ACQ questionnaire is available in Greek translation from (<http://www.qoltech.co.uk>).

Regarding mode of administration, since the ACQ questionnaire contains a range of response options it is more difficult to conduct the questionnaire via a telephone-interview as compared to self-administered ⁽⁴⁷⁾. As for postal questionnaires, it has been suggested that apart from family involvement in the interpretation of questions and responses, there is an increase in error due to missing data ⁽⁴⁷⁾. Therefore, for an accurate estimation of asthma control in children, self-

administered questionnaires during medical consultations in the presence of medical staff/dietician would be most suitable and yield less error.

In comparison, the Childhood Asthma Control Test (cACT) is also a simple and concise questionnaire that can be used to evaluate asthma control in the children aged 5-11 years ⁽⁴²⁾. These self-evaluations quickly determine the level of asthma control and indicate the efficacy of treatment ⁽⁴²⁾. Level of asthma control is categorized into controlled, partly controlled and uncontrolled. The cACT test includes 7 items and has a total score ranging from 0 to 27. The higher the score, the better the asthma control. A score ≥ 20 is defined as 'well-controlled' asthma, <19 'poor' asthma control and ≤ 15 'uncontrolled asthma' ⁽⁴²⁾. Questions consist of night symptoms, need for medication, limitation of activities due to asthmatic attacks, and perception of asthma control as either 'uncontrolled', 'partially-controlled' and 'controlled' ⁽⁴²⁾. Each question has five possible responses: All of the time, most of the time, some of the time, a little of the time and none of the time ⁽⁴²⁾. To the candidate's knowledge, the Child Asthma Control Test (cACT) is not available in the Greek language and use of this questionnaire has not yet been validated in Greek children.

Given the above mentioned rationale, a copy of the translated ACQ for children was requested from Professor Juniper via e-mail (at <http://www.qoltech.co.uk>) and used for the purpose of this study. The original ACQ questionnaire is available in Greek translation and has been validated by Juniper et al in children ⁽⁴³⁾(Appendix 2A 8a/b). Additionally, according to GINA recommendations, as part of patient care, it is crucial that the frequency of unexpected visits to hospital/or physician's clinic and need for hospitalization is assessed ⁽⁴¹⁾. Given that this criteria is not evaluated in asthma control questionnaires, Question 7b was designed for this purpose and included.

Question 7b. *"From your last visit to the asthma specialist, have you had an unexpected visit to the doctor, hospital or emergency? Yes/No*
If yes, how many times?"

Method

At both time-points, the ACQ questionnaire was self-administered by participants assisted by parents in the presence of staff/or candidate. The staff were familiar with the content of the ACQ questionnaire from previous intervention studies.

5.13 Evaluation of asthma-related quality of life in children

Rationale

As mentioned in Chapter 1.5.2, assessment of symptoms and quality of life are not only important in evaluating the efficacy of a therapeutic intervention but also useful in providing valuable information about the disease and its effects on the child and family's daily lives ⁽⁴⁸⁾. The Paediatric Asthma Quality of life Questionnaire (PAQLQ) developed by Juniper measures the quality of life in asthmatic children and adolescents ⁽⁴⁸⁾. This questionnaire has shown good responsiveness, reliability, validity and is able to detect clinical change ^(48; 49). These properties are essential for use in clinical trials ^(48; 49). The PAQLQ is a self or interviewer administered tool for children between 7 and 17 years ^(48; 49). This questionnaire comprises of 23 items which evaluate activity limitations, symptoms and emotional functioning of the child. Children are asked to reflect on how they have been during the previous week and to respond to each of the questions on a 7-point scale (7 = not bothered at all to 1 = extremely bothered). The overall PAQLQ score is the average of all 23 responses. The total score ranges from 1 to 7 where a score of 1 indicates maximum impairment and 7, no impairment ⁽⁴⁸⁾. The mini PAQLQ is a concise version of the original PAQLQ questionnaire that consists of 13 items that measure physical, emotional and social problems experienced by children with asthma ^(43; 50) (Appendix 2A, 9a/b). Children are asked to recall their experiences during the past week and to respond to each question on a scale from 1-7. (1 = severe impairment; 7 = no impairment). The overall score is the mean of the 13 responses ^(43; 50). It has been validated in asthmatic children 6-16 years and is considered to be a reliable responsive measuring tool which is applicable for long-term monitoring in clinical trials ^(49; 50).

Many studies both in asthma and other paediatric conditions, have shown that parents tend to have poor perception in the evaluation of problems that their child experiences as a result of illness ⁽⁵¹⁾. For this reason, the PAQLQ is to be completed by the child. Regarding the language in this tool, it is simple, easy to understand by children older than 7 years ^(48; 50). The only

problem may be the recall time of one week which is sometimes difficult for young children to understand and in this case it is suggested that this questionnaire is administered by trained personnel ^(43; 48). The English version of the PAQLQ and mini PAQLQ are available from www.quoltech.co.uk/Asthma. For the purpose of this study, the mini PAQLQ was translated to Greek by a professional linguistic expert and back-translated (Appendix 2A, 9b).

Methods

Asthma-related quality of life was evaluated using the Greek version of the Mini PAQLQ (Appendix 2A, 9 a/b). Participants assisted by parents/guardians completed the questionnaire in the presence of the candidate/or staff.

5.14 Physical Activity Level

A sedentary lifestyle and intense physical activity have been identified as positive risk factors for asthma development in some children ⁽¹¹⁾. Nevertheless, it has been well-established that regular physical activity improves cardiovascular fitness, self-esteem, co-ordination, bone development, social interaction including reduction of adiposity ⁽⁵²⁾ which outweighs the drawbacks ⁽⁵³⁾. Studies performed in asthmatic children found that regular exercise was beneficial and related to reduced hospital admissions, school absenteeism, medication use, fewer physician consultations, and improved ability to cope with asthma ⁽⁵³⁾.

Physical activity level in children was evaluated as in the ISAAC Environmental Questionnaire (Phase 3) ⁽⁵⁴⁾. Questions 21-22 of the composite questionnaire were used to evaluate frequency of exercise per week, duration and type of sport played (Appendix 2A, 10a/b). In the case where the participant did not engage in any type of sport, the parent was asked why and whether non-participation was due to fear of an asthma attack while playing sport.

Question 21 a. "How many times per week does your child exercise?"

Never/rarely ☐ *1-2 times/week* ☐ *More than or equal to 3 times per week* ☐

Question 21 b. If yes, what type of sport? (for example, swimming, basketball, soccer)

Question 22 a. "For how long does your child play sport hours/day, times/week

Question 22 b. If your child does not play sport for what reason?

Does he/she have an asthma attack during sport? Other

5.15 Biochemical tests and nutritional biomarkers

Rationale

The application of metabolomics in nutrition epidemiology holds great promise and is valuable in deciphering the interactions between diet and health ⁽⁵⁵⁾. The cornerstone method for measuring food intake in epidemiology has been food frequency questionnaires (FFQs). It has been documented that this self-reported instrument is associated with a number of limitations such as underreporting, recall errors, and difficulty in assessment of portion sizes ⁽⁵⁶⁾. These errors can lead to reduced power, underestimated associations and false inferences. Such issues can be overcome by using nutritional biomarkers as objective measures of intake.

In nutritional studies biochemical biomarkers are often used to evaluate nutrient intake of an individual's diet, nutrient status, assess the validity of dietary intake recorded in FFQs, diet histories or 24 hour recalls, and as a method of assessing dietary change and compliance in intervention studies ⁽⁵⁶⁾. Biomarkers provide accurate measures that can be correlated to dietary intake and have less error than dietary intake estimates from conventional nutrition tools ⁽⁵⁶⁾. Also, they do not depend on the respondent's capability to describe foods in detail. Another strength, biomarkers provide a more proximal measure of nutrient status than dietary intake data for disease outcomes and serve as an integrated measure of absorption and metabolism of the nutrient of interest ⁽⁵⁶⁾. They can give an indication of the nutrient status of an individual on a long-term basis unlike most dietary tools which give a 'snapshot' at one point in time ⁽⁵⁶⁾. Combining nutrient estimates from questionnaire data with serologic measures of the same nutrient can provide a powerful tool for estimating the exposure of interest ⁽⁵⁶⁾. Nonetheless limitations to biomarker analysis are: costs, the invasive process itself, changes in one fatty acid may affect the other, not all nutrients have sensitive biomarkers that are measurable and most foods and food groups have no biomarkers. Furthermore, some reflect short-term dietary intake rather than usual diet ⁽⁵⁷⁾. Another possible issue is that biochemical markers are not only influenced by dietary intake alone. Individuals differ in the degree of digestion, absorption and uptake, metabolism and utilization of nutrients. In addition, laboratory errors such as during collection, storage and assays will affect measurements of biomarkers ⁽⁵⁷⁾.

With regards to the application of metabolomics in the study of asthma, previous studies have identified biomarkers to be involved in asthma pathogenesis such as vitamin D ⁽⁵⁸⁾ and omega-3/omega-6 fatty acid ratio ⁽⁵⁹⁾. Findings from observational studies suggest that serum vitamin D deficiency is common in asthmatic children ⁽⁶⁰⁾ and has been associated with increased asthma

incidence ⁽⁶¹⁾, severity ⁽⁶²⁾, poor asthma control ^(63; 64) and reduced lung function ^(63; 64). Simopoulos (2008) documented that omega 6 to omega-3 ratio of 5:1 had a beneficial effect on asthma patients, whereas a ratio of 10:1 adverse consequences ⁽⁵⁹⁾. Also, increased levels of urinary organic acids, threonine, lactate, alanine, carnitine, acetylcarnitine, and trimethylamineN-oxide have been observed during asthma exacerbations suggesting high levels of oxidative stress and lipid peroxidation resulting from inflammation. Contrastingly, levels of acetate, citrate, malonate, hippurate, dimethylglycine, and phenylacetylglutamine seemed to be decreased, compared with the stable condition ⁽⁶⁵⁾.

5.16 Measuring vitamin D status

Plasma concentration of 25(OH)D is the best indicator of overall vitamin D status ^(66; 67). It reflects vitamin D produced subcutaneously and that obtained from food and supplements when sun exposure is minimal and has a circulating half-life of 15 days ^(66; 67). But it is not clear to what extent 25 (OH)D functions as a biomarker of effect relating to health status or asthma outcomes. In contrast, circulating 1,25(OH)₂D is not an accurate biomarker of vitamin D status since it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium and phosphate ⁽⁶⁶⁾. Another drawback is that levels of 1,25 (OH)₂D do not decline until vitamin D deficiency is severe ⁽⁶⁶⁾. There is no universal consensus on levels of serum 25-hydroxyvitamin D ⁽⁶⁸⁾ defining vitamin D deficiency or insufficiency. According to UK and European guidelines, it is current paediatric practice to use a threshold of serum 25-hydroxy vitamin D less than 25 nmol/L (< 10ng/mL) to define vitamin D deficiency and sufficiency > 50 nmol/L (>20ng/mL) ⁽⁶⁸⁾. This is based on the fact that below this threshold the risk of skeletal deformities will become symptomatic ⁽⁶⁷⁾.

Methods:

At both time-points blood and urine tests were undertaken at the Metabolomic clinic located in the city of Athens by trained personnel. The aim of biochemical testing was to determine metabolic profile of participants, dietary biomarkers, validate dietary data and compliance to the dietary intervention. Urine samples were collected at home or on-site at the metabolomics clinic. Participants were instructed that urine samples should be collected in 100 ml sterile urine containers in the morning prior to the breakfast meal and refrigerated until transferred to the clinic or at any time during the day by spot collection at the clinic. Regarding blood samples, participants were able to drink or eat meals up to 2 hours before blood testing. Peripheral blood samples (4 ml) were collected from children with a butterfly needle attached to a syringe and

distributed to vials following a 2-hour fast. The samples were centrifuged, plasma was decanted from the supernatant and stored at -20°C until analysis within 24 h to avoid degradation. In the case of hemolysis, blood collection was repeated. Blood samples were prepared and analysed for plasma fatty acid composition and 25(OH) D whereas organic acids were determined from urine samples.

5.17 Laboratory procedures

a) Serum vitamin D determination

Determination of 25-OH Vitamin D from blood serum was undertaken using Enzyme Immuno Assay (EIA) ⁽⁶⁹⁾. Calibrator (25µL) was added to glass tubes followed by 1mL of 25D-biotin conjugate and the mixture vortexed. Then, 200µL of this mixture was added to the anti-25D antibody coated microtitre plate. The assay mixture was incubated for 2 hours at room temperature and plates were washed to remove unbound 25-D biotin conjugate. Bound 25-D biotin conjugate was identified by adding 200µL Avidin -HRP (Horseradish Peroxidase) for 30 minutes and then the plate was washed again. Tetramethylbenzidine (TMB) substrate was added followed by incubation for 30 minutes and the reaction terminated with acid. The absorbance recorded at 450 nm was inversely proportional to the concentration of 25-OH vitamin D. Serum 25-OH D values were estimated for unknown samples directly from the calibration curve ⁽⁶⁹⁾.

b) Sample preparation for determination of fatty acid composition

The internal standard mixture (200 µL methyl nonadecanoate in hexane containing BHT) was added to 100 mL plasma. Fatty acid (FA) hydrolysis and derivatization into methyl esters was performed by adding 5% v/v methanolic HCl. Transmethylation was performed at 90°C for 60 min. The samples were then brought to room temperature and extraction of FA methyl esters were performed using hexane. They were transferred to GC injection vials with a crimp cap. Mass spectrometry allows direct detection and identification of fatty acids in plasma without affecting quantity or quality, thus lipid extraction before methylation was not included ⁽⁷⁰⁾.

Gas Chromatography-Mass Spectrometry (GC-MS)

The carrier gas used was helium and the sample injection volume was 1 µL. Analysis was performed on an Agilent 6890/5975C GC-MS operating in electron ionization mode. For the

separation of fatty acid methyl esters an HP-5 ms capillary column (30 m x 250 μ m x 0.25 μ m) was used. The initial oven temperature was 70°C, the ramp rate was 4°C/min, and the final temperature was 290°C, held for 4 min. Acquisition was in the scan mode.

Chemicals

Methyl nonadecanoate (74208, Fluka) was used as an internal standard. A mixture of fatty acid methyl esters (47885-U, Supelco) was used for calibration of the standard mixture. All other solvents used were of the highest purity available (methanol (Merck), n-hexane (Merck), HCL (301721, Sigma-Aldrich), 2,6-di-tert-butyl-4-methylphenol (BHT, B1378, Aldrich).

c) Urinary organic acid extraction

Urine samples were stored at -80°C until analysis. Gas Chromatography-Mass Spectroscopy (GC-MS) as previously described by Tanaka et al ⁽⁷¹⁾ was used to identify 34 unique organic acids. Specifically, organic acids were extracted from urine by liquid-liquid extraction after mixing the specimen with an internal standard solution. The oxidation of 2-keto acids with hydroxylamine hydrochloride was performed. Organic acids were converted to their corresponding trimethylsilyl (TMS) ethers with *N,O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (both from Supelco, Bellefonte, PA, USA). The derivatization imparts volatility to the organic acids, which is required for GC-MS analysis. The organic acids-TMS ethers were separated in a capillary gas chromatography column containing an immobilized, non-polar stationary phase. Following chromatographic separation, organic acids were routinely detected by electron impact mass spectrometry performed in the scan mode with a mass range between 50 and 550 m/z. Identification was achieved by comparison to published spectra compounds and quantification by comparison to the calibration of pure standard compounds in ratio to an internal standard. To account for hydration status of patients, metabolites were referenced to creatinine.

NUTRITIONAL METHODS

5.18 Why assess dietary habits in children?

A healthy diet forms the pillars for optimum health, well-being and in the prevention of chronic disease in later life ⁽⁷²⁾. Nurturing healthy dietary habits is especially important in childhood as this is a critical period of growth and development ⁽⁷²⁾. It is well-recognized that dietary habits are moulded from an early age before 5 years old and these habits subsequently become more

difficult to change after adolescence ⁽⁷³⁾. The impact of poor dietary habits on asthma prevalence/incidence was demonstrated by the ISAAC study. As mentioned earlier, children consuming a high fat diet including butter, margarine, burgers and fast food were found to be positively associated with asthma symptoms ^(74; 75; 76). Contrastingly, a healthy diet consisting of fruits, vegetables, eggs, fish, cereals, meat and milk as well as adherence to the Mediterranean diet were inversely associated to asthma ^(74; 75; 76).

The childhood obesity epidemic is a major public health challenge globally ⁽⁷⁷⁾. It has been estimated that in Europe, 1 in 3 eleven-year-olds are overweight or obese ⁽⁷⁷⁾. Children today are growing up in an obesogenic environment that encourages weight gain and obesity. Changes in food preferences (specifically unhealthy, energy-dense, nutrient-poor food choices), availability, affordability and marketing combined with energy imbalance and a decline in physical activity, with more time being spent on screen-based and sedentary leisure activities have contributed to the rise in childhood obesity ⁽⁷⁷⁾. A wide range of psycho-social problems and health complications are linked to childhood obesity including asthma development in children, increased risk of premature onset of illnesses in adulthood (namely diabetes and heart disease), depression, low self-esteem and poor academic performance ^(77; 78).

Recent data from the multi-centred European study, the ENERGY study (European Energy balance Research to prevent excessive weight Gain among Youth) that involved seven European countries Belgium, Greece, Hungary, Netherlands, Norway, Slovenia and Spain, showed that the highest levels of overweight/obese in European school-children (10-12 years) were found in Greece, specifically 44.4% being overweight/obese, of which 11.2% were obese ⁽⁷⁹⁾. In contrast, the lowest levels of overweight/obese were found in Belgium 16.9%, of which 3.7% were obese. Children from Norway and the Netherlands spent on average more than 40 minutes per week cycling to school, whereas children from Greece, on average only up to 7 minutes per week. Breakfast skipping was another dietary behaviour assessed by the researchers. Breakfast skipping in children was more likely with lower parental education in four out of the seven countries considered (Belgium, Greece, Slovenia, and Spain) ^(79; 80).

Given that good dietary habits are learnt during childhood ⁽⁷³⁾ and the importance of educating children about nutrition to optimize health, physical and mental development, as well as in the maintenance of healthy weight and in the prevention of future disease, it is important that health professionals assess dietary habits and rectify poor dietary behaviour that is detrimental to health.

5.19 Measuring dietary intake in children

Rationale

Measuring dietary intake in children enables the assessment of nutritional adequacy, dietary patterns, and intervention compliance as well as provides valuable information about energy intake, food preferences and eating habits ⁽⁸¹⁾. Previous research suggests that collecting reliable and accurate dietary data from this population can be a problem due to reporting bias ⁽⁸²⁾. Parents are often used as proxy reporters of their children's dietary intake ⁽⁸²⁾, since young children < 8 years old have low literacy levels, limited cognitive abilities, cannot accurately recall foods and have difficulties in estimating portion size or frequency of food consumption ⁽⁸²⁾. However, as a child grows older and develops cognitively, the ability to self-report food intake improves ⁽⁸³⁾. The age at which a child becomes an accurate self-reporter of his own dietary intake has been estimated to be approximately 12 years old ⁽⁸³⁾.

Several methods are available to assess individual intakes of foods, nutrients and total energy. Most commonly used are food frequency questionnaires (FFQs), diet history, 24-hour recall, weighed food records and food diaries and biomarkers ⁽⁵⁷⁾. Food diaries are considered to be the gold standard for measuring dietary intake. For this method participants are required to record in detail all foods and drinks consumed on one or more days. In the case of weighed food records, all foods and drinks must be weighed on a kitchen scale, whereas in unweighed records, portion sizes of all foods are estimated using household measures (kitchen measuring utensils), food models or photographs. The latter is more difficult because it requires the participant to be literate, trained for weighing and recording foods in order to ensure accurate results. Ideally, each portion of food should be weighed and recorded before consumption in order to minimize recall bias, although this is time-consuming and places a burden on the participant ⁽⁵⁷⁾.

Another dietary method, the diet history is useful in collecting details on “usual” food consumption patterns ⁽⁵⁷⁾. This method consists of three parts: face-to-face interview, food frequency list and 3-day diet record. Although it collects accurate quantitative data on long-term consumption patterns, it is more costly and time-consuming than FFQs. Similar to the other dietary methods it is subject to recall bias and can be affected by interviewer bias ⁽⁵⁷⁾.

One more dietary tool used in research studies is the 24 hour dietary recall. This is a retrospective method of dietary assessment where an individual is interviewed about their food and beverage intake during the last 24 hour period (i.e on the previous day) ⁽⁵⁷⁾. A key feature of the 24 hour

recall is that the respondent is asked for detailed information regarding time of meals, cooking preparation method, type of food consumed and portion size. Recall of intake over a longer time period is subject to error due to problems in memory recall. The advantage of this method is that it can be interviewer-administered, conducted during face-to-face interviews or by telephone ⁽⁵⁷⁾. This method is quick (requires less than 20 mins), easy to understand/ literacy not required, has low-respondent burden and the procedure does not alter food intake patterns. Previous studies have demonstrated no difference between telephone-administered 24 hour recalls and face-to-face interviews ⁽⁵⁷⁾. Moreover, they may reduce administration costs. A possible limitations of the single 24 hour recall is that it does not represent habitual intake due to day-to-day variation. However, this can be overcome by conducting multiple dietary recalls ⁽⁵⁷⁾. Another drawback might be that this method depends on the respondent's ability to recall intake accurately, estimation of portion size, increase in costs due to interviewer burden especially for repeated recalls ⁽⁵⁷⁾.

Multiple 24 hour dietary recalls were developed for and used by the National Health and Nutrition Examination Survey (NHANES) to assess health, diet and nutritional status in American children and adults ^{(57);(84)}. More specifically, the NHANES study demonstrated that multiple 24-hour recalls can be used to capture an individual's usual dietary intake ⁽⁵⁷⁾. Using multiple 24-hour recalls the diet is examined over a period of three to five days during which the respondent is asked to recall and describe all food and drinks consumed in the 24 hour prior to the interview ⁽⁵⁷⁾. Since variability exists between an individual's macronutrient intake on weekdays and weekends, diet is assessed over 3 consecutive days (2 weekdays and one weekend) using face-to-face or telephone interviews ⁽⁸⁵⁾. The first recall usually takes about 15-20 minutes and the 2nd/3rd recall approximately 10 minutes.

In contrast, FFQs have been widely used in population studies including the NHANES study ^(84; 86) and ISSAC study ⁽⁵⁴⁾ to examine relationships between diet and risk of disease and in measuring long-term dietary intake. FFQs can easily capture usual food intake over the preceding month or year ⁽⁵⁷⁾. An advantage of FFQs is that they are cheap, easy, quick and accurate in recording the individual's usual dietary intake and impose low-burden on participants ⁽⁵⁷⁾. Questionnaires may be useful in assessing nutrient intake, food groups/items such as fruit and vegetables and in assessing change in diet following dietary advice ⁽⁵⁷⁾. In FFQs, respondents are instructed to report their usual frequency of food consumption from a list of foods and beverages during a specific time period ⁽⁵⁷⁾. For each food item, participants must indicate

frequency of consumption by ‘ticking’ one of the frequency categories, ranging from ‘almost never’ to ‘six or more times per day’ ⁽⁵⁷⁾. In semi-quantitative FFQs, portion or serving size information is collected from standardized portions, which is specified as part of the food item ⁽⁵⁷⁾. Respondents are asked to indicate the frequency of consumption of specific quantities of foods based on kitchen measuring tools (e.g ½ cup, 1 cup, 1 teaspoon, 1 tablespoon) or to assess their usual portion size based on food models (small, medium, large) ⁽⁵⁷⁾. As compared to non-quantitative FFQs, semi-quantitative FFQs can provide estimates on nutrient intake. Moreover, in case-control and intervention studies, nutrient intakes calculated from semi-quantitative FFQs are comparable to those from weighed intake records. Nevertheless, whether including portion size in FFQs improves estimation of nutrients, is debatable ⁽⁵⁷⁾. One disadvantage of FFQs is that they lack the detail and specificity of diet records or recalls and may not provide accurate estimates of nutrient intake ⁽⁵⁷⁾. In addition, responders are prone to recall bias, underreporting or over-reporting of foods. Questionnaires may be administered by trained personnel in face-to-face interviews, by telephone or self-administered via postal surveys ⁽⁵⁷⁾.

5.20 Why measure adherence to Mediterranean diet in children?

Rationale

Over the past 20 years, a large body of literature explored and clearly demonstrated the beneficial effects of the Mediterranean dietary pattern for a number of health outcomes ^(87; 88). Early studies undertaken by Trichopoulou showed the prophylactic potential of adherence to the Mediterranean dietary pattern in promoting good health, well-being and longevity ⁽³⁾. The importance of this dietary pattern is related to being a balanced and varied diet and providing most of the recommended macronutrients in their right proportions ^(89; 90). It is characterized by a low content of saturated fatty acids and a high content of monounsaturated fatty acids, fibre and complex carbohydrates including antioxidants that interact synergistically reducing the risk of chronic diseases such as cardiovascular disease, cancer, neurodegenerative diseases, obesity and diabetes in later life ^(88; 91; 92). With respect to allergy prevalence, robust evidence from observational studies suggests that adherence to the Mediterranean dietary pattern reduces asthma prevalence in children ⁽⁹³⁾.

Modernization of society has resulted with the adoption of unhealthy lifestyle habits that have contributed to the rise in child obesity such as modification of food preferences from traditional,

fresh, seasonal food towards “junk” foods and sedentary behaviour leading to an overall imbalance between energy intake and expenditure ⁽⁷⁸⁾. According to the GRECO study, almost one third (29.5%) of school-children (10-12 years) were overweight, 11.7% obese and 46.8% had low adherence to the Mediterranean diet ⁽⁹⁴⁾. Similar data were reported in the PANACEA study, 27.7% of children (10-12 years) were overweight and 6.3% obese with 12.3% reporting high adherence to the Mediterranean diet ⁽⁹⁵⁾. In the IDEFICS study in which 16,220 children aged 2-9 years were recruited from centres in eight European studies, adherence to a Mediterranean-like diet was inversely related to overweight and obesity in children (OR = 0.85, 95% CI: 0.77; 0.94) independent of age, sex, socioeconomic status, study centre and physical activity level ⁽⁹⁶⁾. This is in concordance with other studies documenting an inverse association between the high compliance to the Mediterranean diet and weight status in European children ^(95; 96; 97).

It is unequivocal that diet quality can affect future risk of chronic disease in later life. Good nutrition is important for the physical and mental development of children including optimum health ⁽⁹⁸⁾. Data from observational studies have reported that high adherence to the Mediterranean dietary pattern in children is associated with better diet quality in terms of vitamins and minerals ^(94; 99; 100; 101). Children adhering to the Mediterranean dietary pattern consumed more frequently seafood, fish, legumes, nuts, fruits, leafy vegetables, olives, dairy products, legumes and low glycaemic index foods and unrefined foods ^(94; 100). Given the benefits of the Mediterranean dietary pattern, it is essential that children/adolescents are evaluated frequently in order to rectify poor dietary habits and promote it.

5.21 Adherence to the Mediterranean diet assessment tools

Measuring the effect of adherence to the Mediterranean dietary pattern on disease outcome requires an assessment tool. The most common tools available to assess adherence to this dietary pattern are the Mediterranean diet Score based on Trichopoulou ⁽³⁾, Psaltopoulou ⁽¹⁰²⁾ which is identical to the score devised by Trichopoulou with a minor variation in food groups and the PREDIMED Mediterranean diet assessment tool ⁽¹⁰³⁾. However, these tools have been developed and validated in adult populations. To the candidate's knowledge, no such tool has been designed for Greek children or adolescents. In most paediatric studies, the Mediterranean diet score developed by Trichopoulou ⁽³⁾ Psaltopoulou ⁽¹⁰²⁾ ^(104; 105; 106; 107) as well as the KIDMED score have been used ^(108; 109).

The Mediterranean Diet Score developed by Trichopoulou ⁽³⁾ was originally designed for the adult population in order to examine the effect of adherence to Mediterranean dietary pattern in chronic disease. This scoring system quantifies adherence based on 9 nutritional components (fruit and nuts, vegetables, legumes, cereals, dairy products, fish and seafood, meat and meat products, olive oil, alcohol) of the Mediterranean dietary pattern. Values of 0 or 1 are assigned to each of the components. Persons consuming components with a beneficial effect on health (vegetables, legumes, cereals, fish, fruit and nuts) below the median are assigned a value of 0, whereas those consuming above the median are assigned a value of 1. In contrast, consuming below the median of foods detrimental to health (meat, poultry, dairy, and alcohol) is assigned a value of 1 and consumption above the corresponding is given a value of 0. For fat intake, the ratio of monounsaturated to saturated fats is used since monounsaturated fats (from olive oil) are used in higher quantities than polyunsaturated fats. In the case of alcohol, a value of 1 is given to men whose consumption ranges from 10-50g/day and 5-25g/day for women. Hence, the total Mediterranean-diet score ranges from 0 for minimal adherence to the traditional Mediterranean diet to 9 for maximal adherence ⁽³⁾. Psaltopoulou et al, used the same 10-point Mediterranean diet scale according to Trichopoulou, where a value of 0 denotes minimal adherence to the traditional Mediterranean dietary pattern and 9 maximal adherence ⁽¹⁰²⁾.

In comparison, Garcia- Marcos⁽¹⁰⁶⁾ and Castro Rodriguez⁽¹⁰⁴⁾ used a scoring system based on the Mediterranean diet score used by Psaltopoulou ⁽¹⁰²⁾, but adapted for the Mexican population. Slight modifications were made to the original Mediterranean diet score. Fruit, fish vegetables, legumes, cereals, pasta, rice and potatoes were considered “pro-Mediterranean” foods and rated according to frequency of intake (0 points = never/occasionally; 1 point \geq 1-2 times/week; 2 points \geq 3 times/week). Foods detrimental to health and considered to be “anti-Mediterranean” were meat, milk and fast food which were rated inversely (0 points \geq 3 times/week; 1 point \geq 1-2 times/week; 2 points = never or occasionally). Scores ranged from 0 to 22 in the Garcia-Marcos study and 0-36 in the Castro-Rodriguez study, with a higher score meaning better adherence to the Mediterranean dietary pattern ^(104; 106).

Alternatively, the KIDMED index is a Mediterranean diet quality index designed to assess the diet quality and compliance to the Mediterranean dietary pattern in Spanish children and adolescents participating in the Enkid study ^(108; 109). For the purpose of this thesis study the KIDMED score was deemed most appropriate for the population under investigation. A full description of this tool was given in Chapter 5.8.3 of this thesis.

STATISTICAL METHODS

5.22 Sample size estimation

Rationale

Unlike epidemiological studies which are based on large sample sizes, a smaller number of participants are used in intervention trials, namely due to difficulty in adherence to the interventions, problem of drop out and economic costs ⁽¹¹¹⁾. Nevertheless, the size of the sample must have sufficient statistical power to detect differences in lung function among patients⁽¹¹¹⁾.

Previous RCTs, evaluating the effect of omega-3 fatty acid intake on pulmonary function in children and asthma symptoms have used small samples, medium effect size and were of short duration ^(112; 113; 114; 115). Hodge et al, included 39 children in a six-month RCT ⁽¹¹³⁾ and Nagakura et al, 29 children over a 10-month period in a double-blind RCT ⁽¹¹⁵⁾. A randomized, double blind placebo-self-controlled crossover trial over 38 consecutive weeks consisting of 60 children was conducted by Biltago et al ⁽¹¹²⁾. Similarly, Lee et al, recruited 192 school-children in a 16-week intervention study ⁽¹¹⁴⁾. In the two studies, one undertaken by Lee, (2013) and the other by Biltago (2009), sample size was estimated using differences in means for FEV₁ ^(112; 114). In the study by Biltago et al, a sample size of 60 patients was calculated using 90% power and 5% significance level ⁽¹¹²⁾. Similarly, Lee et al, used a standard deviation for the mean change in FEV₁ of 0.41, and estimated that 102 participants in each group would give a power of 80% at a 5% significance level, which would be large enough to detect a mean difference of 0.16 litres between the two groups for a change in FEV₁ at baseline and at the end of the study ⁽¹¹⁴⁾. Then dividing the mean difference in FEV₁ between the two groups (0.16) by the standard deviation for the change in FEV₁ (0.41) gave an effect size of 0.40.

For the purpose of this research project, the parameters used by Lee (2013) were applied to calculate sample size ⁽¹¹⁴⁾. G Power Statistical Analysis (version 3.2.1) which is a free on-line power analysis program for a variety of statistical tests was used to calculate sample size ⁽¹¹⁶⁾. A priori was selected to calculate sample sizes for powers of at least 80% given that the following parameters were known: number of groups and measurements, effect size, significance level, with two groups being measured at two time points, pre and post intervention (Figure 11).

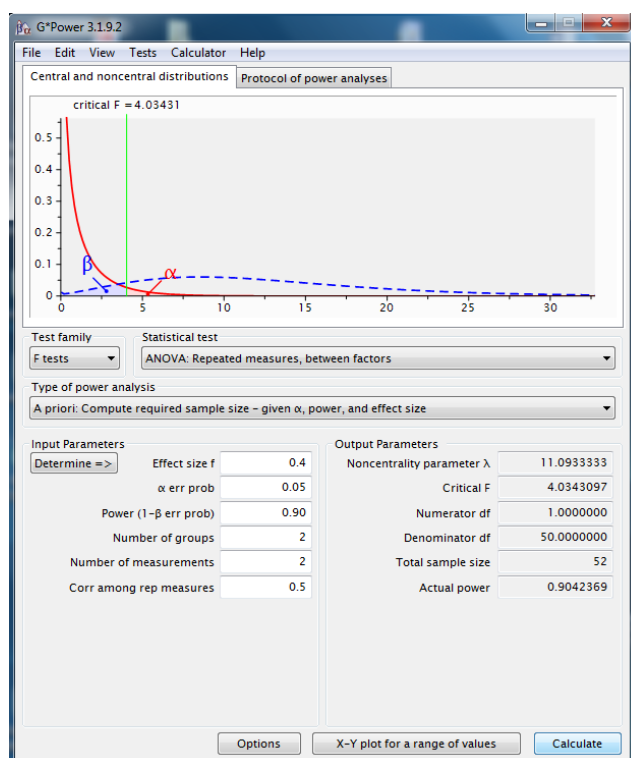


Figure 11 Snap shot of G Power Analysis

Multiple trials were executed using an effect size of 0.4 and 5% significance level until the smallest sample size possible was generated with the highest power (Table 2).

Table 2 G Power Analysis sample size executions

A priori:	Effect size (FEV_1)=0.4, α =0.5, n(groups)=2
Parameters	Statistical family- ANOVA repeated measures between factors Test family-F test, measurements= 2
Power	Sample size (n)
80%	40
85%	46
90%	52
95%	64
97%	72

Therefore, choosing a medium effect size of 0.4 for FEV_1 , 90% power and 5% significance level generated a sample size of **52** participants. Then allowing for a 20% dropout rate produced a **final sample size of 64 participants**, that is 32 participants in each of the two groups.

5.23 Dissemination

Findings of this study were published in scientific journals and presented in international conferences as abstracts, posters and video presentations throughout the four year PhD study. In addition, during November 2018, a media release announcing the main findings of this RCT was conducted by Prof. Itsiopoulos, Prof. Erbas, the candidate and Dr. Tsoukalas that included television, newspapers and online coverage in Australia, Europe and Greece (Please refer to Appendices 3, 4 and 5). In early December 2018, participants at the asthma clinic received from the candidate a brief summary of the study's main findings (Appendix 2A, 19 a/b).

5.24 Research Costs

The candidate is grateful and would like to thank La Trobe University for the financial support provided by La Trobe University Post-graduate Research Scholarship which enabled this project possible and assisted in the payment of project expenses that included printing of questionnaires and associated materials, participant spirometry tests, conference registration fees and production of posters, videos recordings as well as living and travel costs.

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APPENDIX 2C

SAMPLE OF COMPLETED QUESTIONNAIRES AND CLINICAL ASSESSMENTS

Time-point: Baseline

Date: 23.11.2016

Participant No: 15I (Intervention group)

Sex: Male

Age: 6 years old

Assessment tool	
1. Screening Questionnaire (completed by Pneumologist)	533
2. Consent form (signed)	534
3. Socio-demographic Questionnaire	535
4. Asthma Control Questionnaire	539
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6. Dietary Habits Questionnaire/FFQ	543
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12. Record of weekly fatty fish intake	556
13. Spirometry/ FeNO baseline	557
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15. Biochemical Tests (baseline) -Vitamin D	560
16. Biochemical Tests (baseline)- Organic Acids	561
17. Participant Evaluation	564
18. English Translations	567

1. Screening Questionnaire (completed by pneumologist)

Participant ID: 151

2

ΚΡΙΤΗΡΙΑ ΕΙΣΑΓΩΓΗΣ

Ερ.1. Πότε γεννήθηκε το παιδί σας (5-12 ετών): 3/9/2010

Έχει γεννηθεί μεταξύ 2004 και 2011; ΝΑΙ ☒ ΟΧΙ ☐Ερ.2. Έχει ήπιο-μέτριο άσθμα; ΝΑΙ ☒ ΟΧΙ ☐

Αν η απάντηση είναι ΝΑΙ και στις δύο ερωτήσεις τότε θα πρέπει να ελεγχθούν και τα ΚΡΙΤΗΡΙΑ ΑΠΟΚΛΕΙΣΜΟΥ. Αν έστω σε μια από τις δύο ερωτήσεις η απάντηση είναι ΟΧΙ τότε το παιδί δεν έχει δικαίωμα συμμετοχής στη μελέτη.

ΚΡΙΤΗΡΙΑ ΑΠΟΚΛΕΙΣΜΟΥ

Ερ.3. Μήπως το παιδί σας δεν τρώει κανένα από τα εξής λιπαρά ψάρια: σαρδέλα, σολομό, γαύρο, τσιπούρα, κολιό, πέστροφα, σκουμπρί;

ΝΑΙ, δεν τρώει ☐ ΟΧΙ τρώει ☒

Αν, δεν τρώει γιατί;

Είναι αλλεργικό ☐ Δεν του αρέσει κανένα από τα λιπαρά ψάρια ☐ Είναι χορτοφάγο ☐

Ερ.4. Μήπως το παιδί σας έχει κάποιο από τα παρακάτω ιατρικά προβλήματα;

Γαστροφαγική Παλινδρόμηση ΝΑΙ ☐ ΟΧΙ ☒Κυστική Ινωση ΝΑΙ ☐ ΟΧΙ ☒Συγγενή Ανωμαλία αναπνευστικού ΝΑΙ ☐ ΟΧΙ ☒

Ερ.5. Μήπως το παιδί σας παίρνει ιχθυέλαιο συμπλήρωμα;

ΝΑΙ ☐ ΟΧΙ ☒

Αν η απάντηση τουλάχιστον σε μια από τις παραπάνω ερωτήσεις των ΚΡΙΤΗΡΙΩΝ ΑΠΟΚΛΕΙΣΜΟΥ (Ερ.3-Ερ.5) είναι ΝΑΙ τότε το παιδί αποκλείεται από τη μελέτη. Αν σε όλες τις παραπάνω ερωτήσεις των κριτηρίων αποκλεισμού η απάντηση είναι ΟΧΙ τότε το παιδί είναι ΕΠΙΛΕΞΙΜΟ (έχει δικαίωμα συμμετοχής). Αν για το ΕΠΙΛΕΞΙΜΟ παιδί υπογραφεί η φόρμα συγκατάθεσης τότε είναι ΣΥΜΜΕΤΕΧΟΝΤΑΣ και πρέπει να λάβει κωδικό συμμετοχής.

2. Completed Consent form (Participant 151)



LA TROBE
UNIVERSITY

School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

ΣΥΓΚΑΤΑΘΕΣΗ ΕΘΕΛΟΝΤΙΚΗΣ ΣΥΜΜΕΤΟΧΗΣ ΣΕ ΕΡΕΥΝΗΤΙΚΗ ΜΕΛΕΤΗ

Ο/Η ΑΔΑΜΑΝΤΙΑ ΠΑΠΑΔΟΠΟΥΛΟΥ ως κηδεμόνας του/της
ΚΩΝ/ΝΟΥ ΠΑΠΑΔΟΠΟΥΛΟΥ, δηλώνω τη συγκατάθεση μου να συμμετάσχει
στην μελέτη «Η θεραπευτική δράση της Μεσογειακής Διατροφής εμπλουτισμένο με λιπαρά ψάρια σε
παιδιά που πάσχουν από άσθμα» που διεξάγεται από το Πανεπιστήμιο του La Trobe της Αυστραλίας.

Δηλώνω ότι είμαι το υπεύθυνο άτομο για να δώσω συγκατάθεση για την συμμετοχή του/της
ΚΩΝ/ΝΟΥ στη μελέτη.

Γνωρίζω ότι η έρευνα συνίσταται στη συμπλήρωση ερωτηματολογίων και στην κατανάλωση δυο
γεύματων λιπαρών ψαριών ανα εβδομάδα, για 6 μήνες. Οποιαδήποτε πληροφορία ή περαιτέρω
διευκρίνιση θα δοθεί σε μένα και το συμμετέχον τέκνο μου από τους υπευθύνους. Γνωρίζω ότι ο
συμμετέχοντας διατηρεί το δικαίωμα να διακόψει τη συμμετοχή του, όποια στιγμή επιθυμεί χωρίς να
έχει κάποια επίπτωση και χωρίς να απαιτείται να εξηγήσει το λόγο. Επίσης, γνωρίζω ότι όλα τα
προσωπικά στοιχεία, εμού και του τέκνου μου, και τα αποτελέσματα της μελέτης είναι απόρρητα και
θα χρησιμοποιηθούν μόνο για ερευνητικούς σκοπούς.

Έχω διαβάσει το ενημερωτικό φυλλάδιο και υπογράφω με ελεύθερη βούληση.

-----/-----/-----

(υπογραφή γονέα/κηδεμόνα)

Ο Επικ. Καθ. Παιδιατρικής, Χ.Κατσαρδής
Η Υπεύθυνη Καθηγήτρια: Δρ.Κ. Ισιόπουλος
Η Ερευνήτρια : Μαρία.Μ. Παπαμιχαήλ

Υπογραφή:.....
Υπογραφή:.....
Υπογραφή:.....

Κωδικός Συμμετέχοντα/...../...../.....

3.Socio-demographic Questionnaire (Participant 15I)

Ερ1. Τι σχέση έχετε με το παιδί;

Μητέρα ☒ Παππούς ☐
Πατέρας ☐ Γιαγιά ☐
Κηδεμόνας ☐

Ερ2. Τι εθνικότητα έχετε;

Ελληνική ☒ Άλλη ☐

Ερ3. Σε ποιά φυλή θα κατατάσσατε τον εαυτό σας;

Κauκάσιος/α ☒ Μαύρος/η/Αφρικανή ☐ Ασιάτης/α ☐

Ερ4. Ποιά είναι η οικογενειακή σας κατάσταση; Είστε:

Άγαμος (δεν έχετε παντρευτεί) ☐
Έγγαμος (είστε παντρεμένος/η) ☒
Χήρος/χήρα ☐
Διαζευγμένος (έχει εκδοθεί το διαζύγιο) ☐
Σε διάσταση ☐
Με σύμφωνο συμβίωσης ☐

Ερ 5. Με τι ασχολείστε σήμερα; Είστε;

- Άνεργος ☐
- Μισθωτός με πλήρη απασχόληση ☐
- Μισθωτός με μερική απασχόληση ☐
- Ελεύθερος επαγγελματίας ☐
- Δημόσιος υπάλληλος ☐
- Ιδιωτικός υπάλληλος ☒
- Συνταξιούχος ☐
- Έχετε διακόψει την εργασία ή την επιχείρησή σας ☐
- Νοικοκυρά ή φροντίδα παιδιών ☐
- Ακατάλληλος για εργασία ή έχετε μόνιμη αναπηρία ☐

Ερ 6. Τι επίπεδο εκπαίδευσης έχετε τελειώσει;

- | | | | |
|--------------|-------------------------------------|--------------|--------------------------|
| Δημοτικό | <input type="checkbox"/> | ΤΕΙ | <input type="checkbox"/> |
| Γυμνάσιο | <input type="checkbox"/> | ΑΕΙ | <input type="checkbox"/> |
| Λύκειο | <input type="checkbox"/> | Μεταπτυχιακό | <input type="checkbox"/> |
| Κολλέγιο-ΙΕΚ | <input checked="" type="checkbox"/> | Διδακτορικό | <input type="checkbox"/> |

Στοιχεία Συζύγου

Ερ 7. Ο/Η σύζυγός σας τι εθνικότητα έχει;

Ελληνική

☒

Άλλη

.....

☐

Ερ 8. Σε ποιά φυλή θα κατατάσατε τον/την σύζυγό σας;

Κανκάσιος/α

☒

Μαύρος/η/Αφρικανή

☐

Ασιάτης/α

☐

Ερ 9. Ο/Η σύζυγός σας με τι ασχολείται σήμερα; Είναι;

Άνεργος

☐

Μισθωτός με πλήρη απασχόληση

☐

Μισθωτός με μερική απασχόληση

☐

Ελεύθερος επαγγελματίας

☐

Δημόσιος υπάλληλος

☐

Ιδιωτικός υπάλληλος

☒

Συνταξιούχος

☐

Έχετε διακόψει την εργασία ή την επιχείρησή σας

☐

Νοικοκυρά ή φροντίδα παιδιών

☐

Ακατάλληλος για εργασία ή έχετε μόνιμη αναπηρία

☐

Άλλη περίπτωση μη οικονομικού ανέργου ατόμου

☐

Ερ 10. Ο/Η σύζυγός σας τι επίπεδο εκπαίδευσης έχει τελειώσει;

Δημοτικό ☐

ΤΕΙ ☐

Γυμνάσιο ☐

ΑΕΙ ☐

Λύκειο ☐

Μεταπτυχιακό ☐

Κολλέγιο-ΙΕΚ ☒

Διδακτορικό ☐

Ερ 11. Ποιό είναι το οικογενειακό μηναίο εισόδημα σας; (αφορά στο συνολικό εισόδημα, όχι μόνο λόγω εργασίας και ενοίκια;

.....700€.....

Στοιχεία Παιδιού

Ερ 12. Πόσα παιδιά είναι συνολικά στην οικογένεια;

Αριθμός παιδιών στην οικογένεια2.....

Ερ 13. Ποιά είναι η κατάταξη του συμμετέχοντος παιδιού στην οικογένεια;

Πρώτο ☐

Δεύτερο ☒

Τρίτο ☐

Τέταρτο ☐

Ερ 14. Ποιό είναι το φύλο του συμμετέχοντος παιδιού;

Αγόρι ☒

Κορίτσι ☐

Ερ 15. Ποια είναι η ημερομηνία γεννήσεως του συμμετέχοντος παιδιού; 03/09/10

Ερ 16. Τι βάρος και ύψος έχει το παιδί σας; 24,5 Κιλά

1,23 εκ.

Ερ 17. Σε τι είδος σχολείο πηγαίνει το συμμετέχον παιδί; Ιδιωτικό ☐

Δημόσιο ☒

4.Asthma Control Questionnaire (Participant 15I)

2

Ερωτηματολόγιο Παιδιών για τον έλεγχο του Άσθματος

Αυτό το ερωτηματολόγιο να συμπληρωθεί από το παιδί μαζί με τον γονέα/κηδεμόνα.
Σκεφθείτε πως ήσασταν την ΠΕΡΑΣΜΕΝΗ ΕΒΔΟΜΑΔΑ, και βάλτε σε κύκλο τον αριθμό της απάντησης που περιγράφει καλύτερα την κατάσταση σας.

Ερ1. Γενικά, την περασμένη εβδομάδα, πόσες φορές ξυπνούσατε από το άσθμα σας μέσα στη νύχτα;

- ☒ 0 Ποτέ
- 1 Σχεδόν ποτέ
- 2 Λίγες φορές
- 3 Αρκετές φορές
- 4 Πολλές φορές
- 5 Πάρα πολλές φορές
- 6 Δεν μπορούσα να κοιμηθώ λόγω του άσθματος

Ερ2. Γενικά, την περασμένη εβδομάδα, πόσο άσχημα ήταν τα συμπτώματα του άσθματος σας όταν ξυπνούσατε το πρωί (π.χ δύσπνοια, βήχα, σφύριγμα);

- ☒ 0 Κανένα σύμπτωμα
- 1 Πολύ ελαφρά συμπτώματα
- 2 Ελαφρά συμπτώματα
- 3 Μέτρια συμπτώματα
- 4 Αρκετά σοβαρά συμπτώματα
- 5 Σοβαρά συμπτώματα
- 6 Πολύ σοβαρά συμπτώματα

Ερ3. Γενικά, την περασμένη εβδομάδα, πόσο περιορισμένες ήταν οι δραστηριότητές σας λόγω του άσθματος σας (π.χ απουσία από το σχολείο/ή μάθημα);

- ☒ 0 Καθόλου περιορισμένες
- 1 Πολύ λίγο περιορισμένες
- 2 Λίγο περιορισμένες
- 3 Μέτρια περιορισμένες
- 4 Πολύ περιορισμένες
- 5 Υπερβολικά περιορισμένες
- 6 Τελείως περιορισμένες

Ερ4. Γενικά, την περασμένη εβδομάδα, πόσο λαχάνιασμα νιώσατε λόγω του άσθματός σας;

- ☐ 0 Καθόλου
- 1 Πολύ λίγο
- 2 Λίγο
- 3 Μέτριο
- 4 Αρκετό
- 5 Πολύ
- 6 Πάρα πολύ

Ερ5. Γενικά, την περασμένη εβδομάδα, πόσο χρόνο είχατε σφύριγμα στο στήθος;

- ☐ 0 Ποτέ
- 1 Σχεδόν ποτέ
- 2 Λίγο από το χρόνο
- 3 Μέτριο από το χρόνο
- 4 Αρκετό από το χρόνο
- 5 Τον περισσότερο χρόνο
- 6 Συνέχεια

Ερ6. Γενικά, την περασμένη εβδομάδα, πόσες εισπνοές κάνατε κάθε μέρα από το φάρμακο για γρήγορη ανακούφιση (π.χ. Aerolin / Serevent);

(Αν δεν είσατε σίγουρος/η πώς να απαντήσετε αυτή της ερώτησης, παρακαλούμε ζητήστε βοήθεια)

- ☐ 0 Καμιά
- 1 1-2 εισπνοές τις περισσότερες μέρες
- 2 3-4 εισπνοές τις περισσότερες μέρες
- 3 5-8 εισπνοές τις περισσότερες μέρες
- 4 9-12 εισπνοές τις περισσότερες μέρες
- 5 13-16 εισπνοές τις περισσότερες μέρες
- 6 Πάνω από 16 εισπνοές τις περισσότερες μέρες

Pre-bronch
FEV1 = 102 = 0
= 0.02 = 2% well controlled

Ερ7. Απο την τελευταία σας επίσκεψη στο ιατρό, είχατε καμία απρογραμματίστη επίσκεψη στον ιατρό, ή στο νοσοκομείο στα επείγοντα περιστατικά;

ΝΑΙ

☐

ΟΧΙ

☒

Αν, ΝΑΙ πόσες φορές

5. Mini Paediatric Asthma Quality of Life Questionnaire & Physical Activity (Participant 151)

Μίνι Ερωτηματολόγιο Ποιότητας ζωής του Παιδικού Άσθματος

Αυτό το ερωτηματολόγιο να συμπληρωθεί από τον γονέα μαζί με το παιδί.

Παρακαλούμε, για όλες τις ερωτήσεις, κυκλώστε το νούμερο που περιγράφει καλύτερα πως αισθανόσασταν την περασμένη εβδομάδα λόγω του άσθματος σας.

Την περασμένη εβδομάδα, ποσο ενοχληθήκατε από:

	Εξαιρετικά ενοχλημένος /η	Πολύ ενοχλημένος /η	Αρκετά ενοχλημένος /η	Κάπως ενοχλημένος /η	Λίγο ενοχλημένος /η	Ελάχιστα ενοχλημένο/ η	Καθόλου ενοχλημένος /η
Ερ8. Βήχα	1	2	3	4	5	6	7
Ερ9. Αναπνευστικό σφυριγμό	1	2	3	4	5	6	7
Ερ10. Σφίξιμο ή από πόνος στο στήθος	1	2	3	4	5	6	7
Γενικά, πόσο συχνά την τελευταία εβδομάδα νιώσατε:							
Ερ11. Δύσπνοια;	1	2	3	4	5	6	7
Ερ12. Κούραση λόγω των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ13. Να έχετε πρόβλημα στο νυχτερινό ύπνο εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ14. Απελπισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ15. Ανήσυχος ή φοβισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Ερ16. Εκνευρισμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7

	Εξαιρετικά ενοχλημένος /η	Πολύ ενοχλημένος /η	Αρκετά ενοχλημένος /η	Κάπως ενοχλημένος /η	Λίγο ενοχλημένος /η	Ελάχιστα ενοχλημένο/ η	Καθόλου ενοχλημένος /η
Ερ 17. Διαφορετικός ή απομονωμένος εξαιτίας των συμπτωμάτων του άσθματος;	1	2	3	4	5	6	7
Πόσο ενοχλημένος είσασταν την τελευταία εβδομάδα:							
Ερ 18. Κάνοντας γυμναστική (όπως κολύμπι, τρέξιμο, ανέβασμα /κατέβασμα σκάλες ή ποδήλατο);	1	2	3	4	5	6	7
Ερ 19. Έχοντας επαφή με ζώα (π.χ παίζοντας ή φροντίζοντας κατοικίδια ζώα);	1	2	3	4	5	6	7
Ερ 20. Έχοντας δραστηριότητες με την οικογένεια ή με τους φίλους (π.χ τρέχοντας με τους φίλους στο διαλείμμα στο σχολείο);	1	2	3	4	5	6	7

QOL 7 No impairment

Ερ21. Πόσες φορές την εβδομάδα αθλείται το παιδί;

Ποτέ/ σπάνια ☐ Μια-δυο φορές/εβδομάδα ☐ Περισσότερο από τρεις φορές/ εβδομάδα ☒

Αν ΝΑΙ, ποιο άθλημα/ή αθλήματα (π.χ ποδόσφαιρο, μπάσκετ, κολύμπι); ΚΟΛΥΜΠΙ, ΤΑΕ ΚΒΟ ΘΟ
ΕΝΟΡΓΑΤΗ, ΠΟΔΟΣΦΑΙΡΟ

Ερ22. Για πόση ώρα αθλείται συνήθως; 1-2 ώρες/ ημέρα 6 φορές/εβδομάδα

Αν ΟΧΙ, για ποιό λόγο;

Παθαίνει κρίση άσθματος κατά την διάρκεια της άσκησης ☐ ...ΟΧΙ Άλλο ☐

6. Dietary Habits Questionnaire/FFQ (Participant 15I)

Ερωτηματολόγιο Διατροφικών Συνήθειων

Ερ. 1. Το παιδί σας τρώει περισσότερο από μια φορά/εβδομάδα έξω ή φαγητό απ' έξω (π.χ από ψητοπωλείο, μαγαζιά ταχυφαγείας «Goody's»); ΝΑΙ ☐ ΟΧΙ ☒

Ερ. 2. Το παιδί σας πόσες φορές/εβδομάδα τρώει πρωινό; (ΓΑΛΛΑ)

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☒

Ερ. 3. Αν τρώει πρωινό, μήπως τρώει κάποια από τα παρακάτω τρόφιμα και πόσο συχνά;

Δημητριακά ή ψωμί ή φρυγανιές ή τόστ ΟΧΙ ☐ ΝΑΙ ☐ φορές/εβδομάδα

Γαλακτομικά (γάλα, γιαούρτι, τυρί) ΟΧΙ ☐ ΝΑΙ ☒ φορές/εβδομάδα ...7...

Τυρόπιτες ή κρουασάν ή κουλουράκια ή κέικ ΟΧΙ ☐ ΝΑΙ ☒ φορές/εβδομάδα ...1...

Αν ΟΧΙ, τι τρώει συνήθως για πρωινό και πόσες φορές/εβδομάδα;

Ερ.4 Χρησιμοποιείται ελαιόλαδο στη μαγειρική, για το τηγάνισμα και όταν φτιάχνετε γεύμα με μακαρόνια ή ρύζι; ΝΑΙ ☒ ΟΧΙ ☐ Αν ΟΧΙ, τι χρησιμοποιείται;

Οδηγίες:

Για κάθε τρόφιμο, σημειώστε η συχνότητα που καταναλώνει το παιδί σας.

Για παράδειγμα, αν πίνει 1 φλιτζάνι γάλα 2 φορές/ημέρα, βάλετε «1» στην κατηγορία 2-3 φορές/ημέρα.

Αν τρώει 2 φρούτα μια φορά την ημέρα, βάλετε «2» στην κατηγορία 1 φορά/ημέρα

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές /ημέρα	Ισο ή περισσότερο από 4 φορές/ ημέρα
Γάλα (1 φλ.)								
Φρούτα (1 μέτριο ή ½ φλ.)						2	1	

(1 φλ.= 1 φλιτζάνι του καπουτσίνο (240ml), 1 κ.σ= 1 κουταλάκι σούπας, 1 κ.γ = 1 κουταλάκι γλυκού, 1 κομ = 1 κομμάτι, 1 κεσ=1 κεσεδάκι γιαουρτιού (200γρ))

Πόσες μερίδες και φορές τρώει/ ή πίνει το παιδί σας.....;

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές /ημέρα	Ισο ή περισσότερο από 4 φορές/ ημέρα
Ερ 5. Γάλα (1 φλ.)								
Ερ 6. Σοκολατούχο γάλα (1φλ.)	X					1		
Ερ 7. Γιαούρτι (1 κεσ)					1			

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές/ ημέρα	Ισο ή περισσότερο απ 4 φορές/ ημέρα
Ερ 8. Τυρί (άσπρο ή κίτρινο) π.χ φέτα ή κασέρι (40γρ)				1				
Ερ 9. Φρούτα (1μέτριο)								
Ερ 10. Χυμό φρουτών (1 φλ.)						1		
Ερ 11. Λαδερρά π.χ (Μπάμιες, μπριάμ, φασολάκια) (1/2-1 φλ.)	X			1				
Ερ 12. Όσπρια (1 πιάτο-300γρ)			1					
Ερ 13. Δημητριακά π.χ [Πρωινού (1/2 φλ), Ψωμί (1 φέτα=30γ), Φρυγανιές (2 τεμ.)]						1		
Ερ 14. Μακαρόνια (1 φλ. μαγειρεμένο 140 γρ)			1					
Ερ 15. Ρύζι (1 φλ.μαγειρεμένο 160γρ)				1				
Ερ 16. Κρέας κόκκινο π.χ [Χοιρινό/Αρνί/ Μοσχάρι/κατσίκι/ μπιφτέκια (2 τεμ)] (150γρ μαγειρεμένο= μερίδα εστιατορίου)				1				
Ερ 17. Κρέας λευκό π.χ [Κοτόπουλο/ κουνέλι/ Γαλοπούλα] (150γρ)			1					
Ερ 18. Παστίτσιο/ Μουσακά/ Μακαρόνια με κιμά/ Παπουτσάκια/ Γεμιστά (150γρ μερίδα εστιατορίου)			1					
Ερ 19. Θαλασσινά π.χ [καλαμάρι, σουπιές, γαρίδες, μύδια, οχταπόδι] (150γρ =μερίδα εστιατορίου)	X		1					
Ερ 20. Ψάρι (2% λιπαρά) π.χ [Σαργός, Γόπες, Μαρίδα, Αθερίνα, Φαγρί, Μπακαλιάρος, Γλώσσα, Λιθρίνι, Ξιφίας, Λαβράκι Μπαρμπούνι, Τόνο Κουτσομούρες] (150γρ =μερίδα εστιατορίου)			1					

Τρόφιμο (μερίδα)	Ποτέ/ Σπάνια	1-3 φορές/ μήνα	1 φορά/ εβδομάδα	2-3 φορές/ εβδομάδα	4-6 φορές/ εβδομάδα	1 φορά/ ημέρα	2-3 φορές/ ημέρα	Ισο ή περισσότερο από 4 φορές/ ημέρα
Ερ 21. Ψάρι λιπαρό π.χ [Σαρδέλα/Γαύρο (12 κομ.), Κολίος, Σολωμός Σκουμπρί, Πέστροφα, Τσιπούρα] (150γρ)			1					
Ερ 22. Μαργαρίνη(1 κ.γ)				1				
Ερ 23. Ξηρούς Καρπούς (1 χούφτα/1/3 φλ./ 50γρ)	X							
Ερ 24. Ελαιόλαδο (1κ.σ)						1		
Ερ 25. Φαγητό Ταχυφαγείας π.χ [Χάμπουργερ (1 τεμ), Σουβλάκι (1 τεμ), Πίτσα (2 κομ.), Χοτ Ντογ (1 τεμ)]		1						
Ερ 26. Πίτες π.χ [Τυρόπιτες, Κρουσάν, Σπανακόπιτες] (1 τεμ = 150γρ)		1						
Ερ 27. Γλυκά π.χ [Πάστες (1τεμ.), Κουλουράκια (2 τεμ), Μπισκότα (2 τεμ), Κέικ (1 κομ.), Κρουσάν (1 τεμ.), Παγωτά (1 μπάλα), Μιλκσεικ (1 φλ), Σοκολάτα (60γρ)]					1			
Ερ 28. Αλμυρά σνάκ π.χ [Πατατάκια, Γαριδάκια, πόπκορν] (1 πακέτο 70γρ)				1				
Ερ 29. Αναψυκτικά/ Αθλητικά ποτά (1 κουτάκι 220 ml)		1						
Ερ 30. Λαχανικά βραστά (π.χ μπρόκολο, λάχανο, κουνουπίδι, χόρτα,κολοκύθια, σπανάκι, παντζάρια) (1/2-1 φλ)	X							
Ερ 31. Σαλάτα ωμή (π.χ μαρούλι, λάχανο, ρόκα, καρότα, <u>τομάτα</u> , αγγούρι)(1/2-1 φλ.)				1				

Σας Ευχαριστούμε πολύ

7. Medical History Questionnaire (Participant 15I)

Πληροφορίες για τη μητέρα

Ερ1. Καπνίζατε κατά τη διάρκεια της εγκυμοσύνης; ΝΑΙ ☒ ΟΧΙ ☐

Ερ2. Καπνίζατε κατά τη διάρκεια του πρώτου έτους της ζωής του παιδιού; ΝΑΙ ☒ ΟΧΙ ☐

Ερ3. Καπνίζετε σήμερα; ΝΑΙ ☒ ΟΧΙ ☐

Αν ΝΑΙ, ποιά μάρκα;

Αν ΝΑΙ, πόσα τσιγάρα/ημέρα; ☐ ή πόσα πακέτα/ημέρα ☐ ;

Ερ4. Όταν ήσασταν μικρή (κάτω από 16 ετών) είχατε: ΝΑΙ ☐ ΟΧΙ ☒

Ασθμα; ☐

Ρινίτιδα; ☐

Εκζεμα; ☐

Ερ5. Στην ενήλικη ζωή (16 ετών έως σήμερα) είχατε: ΝΑΙ ☐ ΟΧΙ ☒

Ασθμα; ☐

Ρινίτιδα; ☐

Εκζεμα; ☐

Πληροφορίες για τον πατέρα

Ερ6. Ο πατέρας κάπνιζε κατά τη διάρκεια του πρώτου έτους της ζωής του παιδιού; ΝΑΙ ☐ ΟΧΙ ☒

Ερ7. Ο πατέρας καπνίζει σήμερα; ΝΑΙ ☐ ΟΧΙ ☒

Αν ΝΑΙ, ποιά μάρκα;

Αν ΝΑΙ, πόσα τσιγάρα/ημέρα; ☐ ή πόσα πακέτα/ημέρα ☐ ;

Ερ8. Ο πατέρας όταν ήταν μικρός (κάτω από 16 ετών) είχε: ΝΑΙ ☐ ΟΧΙ ☒

Ασθμα; ☐

Ρινίτιδα; ☐

Εκζεμα; ☐

Ερ9. Ο πατέρας στην ενήλικη ζωή (16 ετών έως σήμερα) έχει: ΝΑΙ ☐ ΟΧΙ ☒

Ασθμα; ☐

Ρινίτιδα; ☐

Εκζεμα; ☐

Πληροφορίες για παιδιά

Ερ10. Πόσες εβδομάδες διήρκησε η κύηση μέχρι τον τοκετό;

32-37 εβδομάδες ☐

37-40 εβδομάδες ☒

>40 εβδομάδες ☐

Ερ11. Τι βάρος είχε το παιδί όταν γεννήθηκε;

< 2500 γραμμάρια ☐

2500- 4000 γραμμάρια ☒

>4000 γραμμάρια ☐

Ερ12. Το παιδί γεννήθηκε με:

Φυσιολογικό τοκετό ☐

Καισαρική τομή ☒

Ερ13. Θηλάσατε το παιδί όταν ήταν μωρό; ΝΑΙ ☒ ΟΧΙ ☐

Αν ΝΑΙ, για πόσο καιρό;

<3 μήνες ☐

3-6 μήνες ☐

6-12 μήνες ☒

>12 μήνες ☐

Ερ14. Σε ποιά ηλικία εμφανίστηκε το άσθμα του παιδιού;ετών

Ερ15. Το παιδί πάσχει από αλλεργίες; ΝΑΙ ☒ ΟΧΙ ☐

Αν ΝΑΙ, τότε πάσχει απο

Ρινίτιδα/ εποχιακή ρινίτιδα; ☒

Επιπεφυκίτιδα; ☐

Έκζεμα; ☒

Τροφική αλλεργία; ☐

Αν, Ναι σε τι;ετών

Ερ16. Το παιδί τον τελευταίο μήνα βρίσκεται σε φαρμακευτική αγωγή; ΝΑΙ ☒ ΟΧΙ ☐

Αν ΝΑΙ, κάθε πότε;

Καθημερινά βάση θεραπείας; ☒ Για πόσο καιρό; εβδομάδες






Μόνο όταν έχει επεισόδιο; ☐ Πόσες φορές είχε επεισόδια; φορές







Όταν είναι άρρωστος; ☐ Πόσες φορές ήταν άρρωστος; φορές






1x Singular / βρεΐου



Aerolin } στην ΑΡΡ-ΣΤΑΝΔΑ
 Flixotide }

Ερ17. Τι φάρμακα λαμβάνει και με ποιά συχνότητα;

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ		ΔΟΣΟΛΟΓΙΑ	
ΒΡΟΓΧΟΔΙΑΣΤΑΛΤΙΚΑ			
Aerolin (100 µg)		<input type="checkbox"/>φορές/ημέρα
ΑΝΤΙΦΛΕΓΜΟΝΩΔΗ ΚΟΡΤΙΚΟΕΙΔΗ		ΔΟΣΟΛΟΓΙΑ	
Flixotide (125 µg)		<input type="checkbox"/>φορές/ημέρα
Flixotide (250 µg)		<input type="checkbox"/>φορές/ημέρα
Seretide (125 µg)		<input type="checkbox"/>φορές/ημέρα
Seretide (250µg)		<input type="checkbox"/>φορές/ημέρα

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
Seretide (Discus) (100μg)		<input type="checkbox"/>φορές/ημέρα
Seretide (Discus) (250μg)		<input type="checkbox"/>φορές/ημέρα
Symbicort (80μg)		<input type="checkbox"/>φορές/ημέρα
Symbicort (160μg)		<input type="checkbox"/>φορές/ημέρα
ΑΝΤΙΑΕΥΚΟΤΡΙΕΝΕΣ			ΔΟΣΟΛΟΓΙΑ
Singulair (Montelukast) (5mg)		<input checked="" type="checkbox"/> ¹ φορές/ημέρα 1 χάπι / βράδυ Νοβ - Ιούνιο
Miralust (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
Apilone (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
Modulair (Montelukast) (5mg)		<input type="checkbox"/>φορές/ημέρα
KOPTIZONH (per os)			ΔΟΣΟΛΟΓΙΑ
Medrol per os (16mg) (Prednisolone)		<input type="checkbox"/>φορές/ημέρα
Prezolon (5mg)		<input type="checkbox"/>φορές/ημέρα
Soldesanil drops		<input type="checkbox"/>φορές/ημέρα
PINITIDA			ΔΟΣΟΛΟΓΙΑ
Nasonex		<input type="checkbox"/>φορές/ημέρα
Mometasone nasal spray		<input type="checkbox"/>φορές/ημέρα
Pulmicort nasal		<input type="checkbox"/>φορές/ημέρα

ΕΙΔΟΣ ΦΑΡΜΑΚΩΝ			ΔΟΣΟΛΟΓΙΑ
ANTI-ΙΣΤΑΜΙΝΙΚΑ			
Aerius (σιρόπι) Aerius (ταμπλέτες)		<input type="checkbox"/>φορές/ημέρα
Χοζαλ (σιρόπι) Χοζαλ (ταμπλέτες)		<input type="checkbox"/>φορές/ημέρα

Ερ18. Το παιδί λαμβάνει ;

Συμπληρώματα διατροφής; ☐ Βιταμίνες; ☐ Τίποτα απο τα δύο; ☒

Αν ΝΑΙ, ποιο ; πόσα χάπια/ημέρα.....

8. KIDMED Questionnaire (Participant 15I)

15I *Παριφορέας*

Όνομα *Παπαδόπουλος Κων/νος* KIDMED Score *26/11/16*
Κιόνος 4

Παρακαλώ απαντήστε με «X» για τα παρακάτω ερωτήσεις:

Το παιδί σας;

Ερ 1. Τρώει ένα φρούτο ή πίνει ένα χυμό κάθε μέρα; NAI ☒ OXI ☐

Ερ 2. Τρώει δύο φρούτα κάθε μέρα; NAI ☒ OXI ☐

Ερ 3. Τρώει σαλάτα ή βραστά λαχανικά μια φορά την ημέρα; NAI ☐ OXI ☒

Ερ 4. Τρώει σαλάτα ή βραστά λαχανικά περισσότερο από μια φορά την ημέρα; NAI ☐ OXI ☒

Ερ 5. Τρώει ψάρι συχνά (2-3 φορές/εβδομάδα); NAI ☐ OXI ☒ *1x/εβδ*

Αν Ναι, πόσο ψάρι ανα γεύμα;

60-90γ μαγειρεμένα * ☐ 90-120γ ☒ 120-150 γ ☐ Περισσότερο από 150γρ ☐

*Η ποσότητα αναφέρεται στο βάρος του μαγειρεμένου ψαρι χωρίς το κεφάλι και κόκκαλα.

Τι είδος ψάρι τρώει συνήθως π.χ σαρδέλα, τσιπούρα, μπακαλιάρο
σαρδέλα, τσιπούρα

Ερ 6. Τρώει φαγητό απ' έξω (π.χ χάμπουργερ, σουβλάκι) περισσότερο από μια φορά την εβδομάδα; NAI ☐ OXI ☒ *1-2x/μην* *σουβλάκι, πίτσα*

Ερ 7. Τρώει όσπρια περισσότερο από μια φορά/εβδομάδα; NAI ☐ OXI ☒ *1x/εβδ* *φακές*

Ερ 8. Τρώει μακαρόνια ή ρύζι σχεδόν κάθε μέρα (περισσότερο από 5 φορές την εβδομάδα); NAI ☐ OXI ☒ *2-3x/εβδ*

Ερ 9. Τρώει δημητριακά ή ψωμί για πρωινό; NAI ☐ OXI ☒

Ερ 10. Τρώει γαλακτομικά (π.χ γάλα, γιαούρτι, ή τυρί) για πρωινό; NAI ☒ OXI ☐

Ερ 11. Τρώει πίτες (π.χ τυρόπιτες, κουλουράκια) ή κρουσάν για το πρωινό; NAI ☐ OXI ☒

Ερ 12. Παραλείπει το πρωινό γεύμα; NAI ☐ OXI ☒

Ερ 13 Τρώει ξηρούς καρπούς συχνά (τουλάχιστον 2-3 φορές/εβδομάδα); NAI ☐ OXI ☒

Ερ 14 Τρώει 2 γιαούρτια και/ή τυρί (40γρ) κάθε μέρα; NAI ☒ OXI ☐ *1 για (40γ) + 1 τυρί*

Ερ 15. Τρώει γλυκά και καραμέλες πολλές φορές κάθε μέρα; NAI ☒ OXI ☐

Ερ 16. Χρησιμοποιείται ελαιόλαδο στη μαγειρική στο σπίτι; NAI ☒ OXI ☐

Κάθε μέρα ωστόσο ή μηνιαία

9. 24 hr Dietary Recall 1 Baseline (Participant 15I) (Saturday)



Παρεσόντας Κων/νος



METABOLOMIC MEDICINE®
HEALTH CLINIC FOR AUTOIMMUNE AND ENDOCRINE DISEASES



Βασιλόπουλος
...και του πουλιού το γάλα!

ΣΚΛΑΒΕΝΙΤΗΣ

Ημερομηνία: 26/11/16 (Σάββατο)

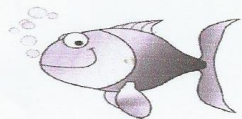
Κωδικός συμμετέχοντα: 15I

ΑΝΑΚΛΗΣΗ 24 ώρου 1 (Baseline)

Συμπληρώστε τι έφαγε το παιδί σας χθές.

ΓΕΥΜΑΤΑ	ΤΡΟΦΙΜΟ	ΠΟΣΟΤΗΤΑ	ΤΡΟΠΟΣ ΜΑΓΕΙΡΕΜΑΤΟΣ
ΠΡΩΙΝΟ	1 γάλα 4%	1 καύτα (250ml)	
ΔΕΚΑΤΙΑΝΟ	1 τρώγ + 3ος τυρί + 3ος τα βράδια		
ΜΕΣΗΜΕΡΙΑΝΟ στο σχολείο	Μακαρόνια + 2 κ.σ. νρι.	Τυρί 1 μερίδα 3ος	
ΑΠΟΓΕΥΜΑΤΙΝΟ	ψωμί 2 φέτες (6ος) + 1 φράιτο (μπανάνα)	ΜΕΡΟΥΔΑ (1 κ.σ)	
ΒΡΑΔΥΝΟ	γάλα 4% (250ml) + Κορνφλέις (1 φ.σ) με σουδάδα		
ΠΡΟ-ΥΠΝΟΥ			

10. 24 hr Dietary Recall 2 (Participant 15I) 3-Months (Friday)



15I Intervention Group

Παπαδόπουλος Κωνσταντίνος

METABOLIC MEDICINE
HEALTH CLINIC FOR AUTOIMMUNE AND CHRONIC DISEASESΒασιλόπουλος
...και του παιδιού το γάλα!

ΣΚΛΑΒΕΝΙΤΗΣ

Ημερομηνία: 17/2/17 Παρασκευή

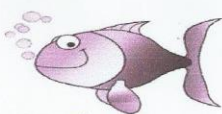
Κωδικός συμμετέχοντα: 15I

ΑΝΑΚΛΗΣΗ 24 ώρου 2 (3 φορές)

Συμπληρώστε τι έφαγε το παιδί σας χθές.

ΓΕΥΜΑΤΑ	ΤΡΟΦΙΜΟ	ΠΟΣΟΤΗΤΑ	ΤΡΟΠΟΣ ΜΑΓΕΙΡΕΜΑΤΟΣ
ΠΡΩΙΝΟ κάθε μέρα	1 ψάδι 4%	2 ποντ	
ΔΕΚΑΤΙΑΝΟ	1 Croissant με σοκολάτα		
ΜΕΣΗΜΕΡΙΑΝΟ	Πουρρεδέκια 3 τεμ Καρύδα 1		(όχι λαχανικά) (όχι σαλάτα)
ΑΠΟΓΕΥΜΑΤΙΝΟ	1 τσίτσι (60g) + 30g τυρί + 30g μαζουράδα + 2 τρ 1 κύμα ΑΛΙΜ (250ml)		φαίκελα
ΒΡΑΔΥΝΟ	2 καλαμάκια	χοιρίνα + 2 φέτες ψωμί (60g)	
ΠΡΟ-ΥΠΝΟΥ	—	—	—

11. 24-hr Dietary Recall 3 (Follow-up) (Participant 15I) (Saturday)


 Παπαδόπουλος Κωνσταντίνος 15I
 (follow up)

Ημερομηνία: 20/5/17

Ημερομηνία: 20/5/17

Κωδικός συμμετέχοντα: 15I

ΑΝΑΚΛΗΣΗ 24 ώρου
 Συμπληρώστε τι έφαγε το παιδί σας χθές.

ΓΕΥΜΑΤΑ	ΤΡΟΦΙΜΟ	ΠΟΣΟΤΗΤΑ	ΤΡΟΠΟΣ ΜΑΓΕΙΡΕΜΑΤΟΣ
ΠΡΩΙΝΟ κασέρφι.	1 φλ. γάλα	> 300ml	
ΔΕΚΑΤΙΑΝΟ	1 κωστίτσα	"ΑΜΑΡΕΤΤΙ"	7ος 1 τεμ.
ΜΕΣΗΜΕΡΙΑΝΟ	παστίτσιο + ντομάτα σαλάτα + 30g ψωμί + τυρί (60g)	1 μερίδα επαγορίου.	
ΑΠΟΓΕΥΜΑΤΙΝΟ	1 μπακλαβά		
ΒΡΑΔΥΝΟ	παστίτσιο	1 μερίδα επαγορίου	
ΠΡΟ-ΥΠΝΟΥ	1 γάλα	> 300ml	

12. Record of weekly fatty fish intake (Participant 151)

Κωνσταντίνος Παπαδόπουλος

ΟΔΗΓΙΕΣ: Παρακαλώ να καταγράφετε κάθε εβδομάδα τις δύο ημέρες που το παιδί σας καταναλώνει λιπαρό ψάρι και την ποσότητα που καταναλώνει κάθε φορά.

Για παράδειγμα, αν καταναλώνει την πρώτη εβδομάδα, ημέρα Τρίτη 150γρ. γαύρο (15 ψαράκια) και το Σάββατο 200γρ. πέστροφα (μαγειρεμένο χωρίς το κεφάλι και κόκκαλα), θα καταγράψετε στην Εβδομάδα, ημέρα Τρίτη 150γρ. γαύρο και στη ημέρα Σάββατο 200γρ. πέστροφα. (βλ. παράδειγμα σε πράσινο φόντο) Έκανε έρευνα αλληλ-συρρα Πέμπτη 3/12/16

ΗΜΕΡΟΜΗΝΙΑ ΕΝΑΡΞΗΣ ΚΑΤΑΝΑΛΩΣΗΣ ΛΙΠΑΡΩΝ ΨΑΡΙΩΝ	ΚΑΤΑΓΡΑΦΗ ΚΑΤΑΝΑΛΩΣΗΣ ΛΙΠΑΡΩ ΨΑΡΙ						
	2 φορές/εβδομάδα τουλάχιστον 150γ μαγειρεμένο ψάρι ανα γεύμα						
ΕΒΔΟΜΑΔΑ	ΔΕΥΤΕΡΑ	ΤΡΙΤΗ	ΤΕΤΑΡΤΗ	ΠΕΜΠΤΗ	ΠΑΡΑΣΚΕΥΗ	ΣΑΒΒΑΤΟ	ΚΥΡΙΑΚΗ
Παράδειγμα		150γ γαύρο (15 ψαράκια)				200γ πέστροφα	
ΕΒΔΟΜΑΔΑ 1						15 γαρίκια σαρόλα	15 γαρίκια
ΕΒΔΟΜΑΔΑ 2		150γρ γολομό		150γρ γολομό			
ΕΒΔΟΜΑΔΑ 3	150γρ τσιπούρα				150γρ γολομό		
ΕΒΔΟΜΑΔΑ 4		150γρ τσιπούρα					150γρ γολομό
ΕΒΔΟΜΑΔΑ 5			15 γαρίδες				150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 6		150γρ τσιπούρα				15 γαρ γαρίδες	
ΕΒΔΟΜΑΔΑ 7			150γρ γολομό			150γρ γολομό	
ΕΒΔΟΜΑΔΑ 8		15 γαρίκια σαρόλα				150γρ γολομό	
ΕΒΔΟΜΑΔΑ 9		150γρ τσιπούρα				20 γαρίκια γαύρο	
ΕΒΔΟΜΑΔΑ 10			150γρ γολομό				150γρ γολομό
ΕΒΔΟΜΑΔΑ 11			150γρ τσιπούρα				150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 12				150γρ γολομό			150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 13			150γρ τσιπούρα				150γρ γολομό
ΕΒΔΟΜΑΔΑ 14			150γρ γολομό				150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 15			150γρ τσιπούρα				150γρ γολομό
ΕΒΔΟΜΑΔΑ 16			150γρ γολομό				150γρ γολομό
ΕΒΔΟΜΑΔΑ 17				150γρ τσιπούρα			150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 18			15 γαρ γαρίδες				15 γαρίδες
ΕΒΔΟΜΑΔΑ 19			150 γαρίδες				150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 20			15 γαρίδες				150γρ τσιπούρα
ΕΒΔΟΜΑΔΑ 21			15 γαρίδες				15 γαρίδες
ΕΒΔΟΜΑΔΑ 22		150γρ γολομό				150γρ γολομό	
ΕΒΔΟΜΑΔΑ 23		150γρ γολομό				150γρ γολομό	
ΕΒΔΟΜΑΔΑ 24		150γρ γολομό				15 γαρ γαρίδες	
Ημερομηνία λήξης στις 6 μήνες							

17/17

13. Spirometry/ FeNO/ Pulse Oximetry (Participant 15I)

Pulmonary Function Test Results

15I HbO₂ 99% FeNO 7 ppb

CHARIS KATSARDIS

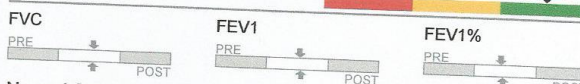
ASS. PROF. OF PED. PULMONOLOGY

PAFOU 1, MAROUI - 210 93.22.946

Visit date 23/11/2016

Patient code 1499
 Surname ΠΑΠΑΔΟΠΟΥΛΟΣ Age 6
 Name ΚΩΝ/ΝΟΣ Gender Male
 Date of birth 3/9/2010 Height, cm 127
 Ethnic group Caucasian Weight, kg 24
 Smoke No smoker BMI 14,88
 Patient group Pack-Year

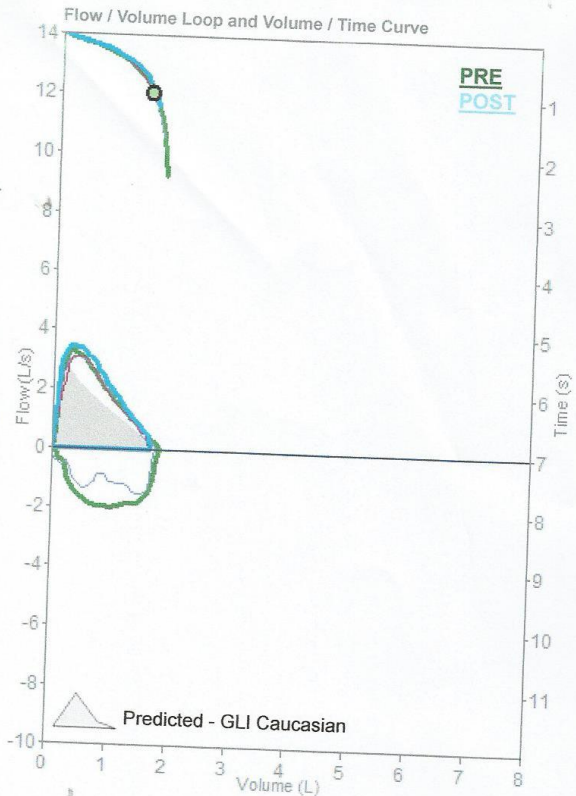
Interpretation



Normal Spirometry
 No Significant Bronchodil.
 WARNING: CHG FEF2575 = 36%

Best values from all loops

Parameters	LLN	ULN	PRE	%Pred	Z-score	POST	%Chg
FVC L	1,38	2,12	1,80	103	0,22	1,80	0
FEV1 L	1,22	1,87	1,57	102	0,13	1,59	1
FEV1% %	77,3	100,6	87,20	98	-0,29	88,30	1
PEF L/s	0,16	5,60	3,32	115		3,51	6



PRE Trial date 23/11/2016 8:05:48 μμ

Parameters	LLN	ULN	Pred	PRE # 1	%Pred	Z-score	PRE # 2	PRE # 3	POST#1	%Pred	%Chg
FVC L	1,38	2,12	1,75	1,80	103	0,22	1,72	1,59	1,65	94	-8
FEV1 L	1,22	1,87	1,54	1,57	102	0,13	1,53	1,55	1,59	103	1
FEV1/VC %	77,3	100,6	88,9	88,9							
FEV1/FVC %	77,3	100,6	88,9	87,2	98	-0,29	89,0	97,5	96,4	108	11
PEF L/s	0,16	5,60	2,88	3,32	115		3,10	3,13	3,51	122	6
FEF2575 L/s	1,16	2,62	1,89	1,76	93	-0,26	2,06	2,09	2,40	127	36
ELA Years			6								
FET s			6,00	2,37	40		1,76	1,16	1,20	20	-49
EVol mL				50			60	80	40		-20
FIVC L	1,38	2,12	1,75	1,72	98	-0,14	1,76				

BTPS 1,115 20 °C 68 °F

Conclusion / Medical report

Quality Report


D

Repeatable FVC, Repeatable
 FEV1, Repeatable PEF

Signature

Instrument used
 Spirobank II S/N 004488

14. Biochemical tests Baseline (Participant 15I): Fatty Acid Composition



Neolab

Dr. Dimitris Tsoukalas, MD
Chronic Diseases & Metabolic Disorders Clinic
Harvard Medical School Course in Gen. Internal Medicine Graduate
President of The European Institute of Nutritional Medicine
American College for the Advancement in Medicine

Ω3 L
VIT-DL

Δρ. Α. Μυλωνά, Δρ Ε. Παραμέρα, Δρ Γ. Ζαχαριουδάκης, Δ. Κατακουζηνός, Κ. Δουλή

Όνοματεπώνυμο : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ

Ημ/νία Εξέτασης : 02/12/2016

ΟΛΙΚΑ ΛΙΠΑΡΑ ΟΞΕΑ ΣΤΟ ΠΛΑΣΜΑ

Εξέταση	Αποτέλεσμα	Φυσιολογικές Τιμές
ΠΟΛΥΑΚΟΡΕΣΤΑ Ω3		
α-Linolenic(C18:3 ω3)	7.73 μmol/L	30.00 - 70.00
Eicosatrienoic(C20:3ω3)	4.00 μmol/L	
Eicosapentanoic(C20:5ω3)	24.61 μmol/L	15.00 - 95.00
Docosahexaenoic(C22:6ω3)	40.37 μmol/L	75.00 - 180.00
ΠΟΛΥΑΚΟΡΕΣΤΑ Ω6		
γ-Linolenic(C18:3 ω6)	7.31 μmol/L	15.00 - 50.00
Linoleic(C18:2ω6)	505.23 μmol/L	1950.00 - 3500.00
Arachidonic(C20:4ω6)	309.76 μmol/L	300.00 - 650.00
Homo-γ-linolenic(C20:3ω6)	82.32 μmol/L	70.00 - 190.00
ΜΟΝΟΑΚΟΡΕΣΤΑ-ΠΟΛΥΑΚΟΡΕΣΤΑ		
Myristoleic(C14:1)	1.13 μmol/L	0.00 - 10.00
Cis-10 pentadecenoic(C15:1)	14.70 μmol/L	
Palmitoleic(C16:1ω7)	45.37 μmol/L	85.00 - 330.00
Oleic(C18:1ω9 cis)	451.93 μmol/L	1035.00 - 2025.00
Elaidic(C18:1ω9 trans)	μmol/L	
Cis-11 Eicosenoic(C20:1ω9)	3.02 μmol/L	10.00 - 25.00
Erucic(C22:1ω9)	1.70 μmol/L	0.00 - 8.00
Nervonic(C24:1ω9)	36.29 μmol/L	55.00 - 85.00
ΚΟΡΕΣΜΕΝΑ		
Octanoic(C8)	μmol/L	
Decanoic(C10:0)	μmol/L	
Undecanoic(C11:0)	μmol/L	
Lauric(C12:0)	5.00 μmol/L	
Myristic(C14:0)	28.97 μmol/L	50.00 - 145.00
Pentadecanoic(C15:0)	7.43 μmol/L	
Palmitic(C16:0)	1296.79 μmol/L	1465.00 - 2790.00
Heptadecanoic(C17:0)	9.99 μmol/L	
Stearic(C18:0)	383.90 μmol/L	465.00 - 755.00

14. Biochemical tests (Participant 15I): Fatty Acid Composition

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Όνοματεπώνυμο : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ Ημ/νία Εξέτασης : 02/12/2016

ΟΛΙΚΑ ΛΙΠΑΡΑ ΟΞΕΑ ΣΤΟ ΠΛΑΣΜΑ

Εξέταση	Αποτέλεσμα	Φυσιολογικές Τιμές
Arachidic(C20:0) :	9.48 μmol/L	15.00 - 30.00
Behenic(C22:0) :	19.07 μmol/L	40.00 - 100.00
Lignoceric(C24:0) :	15.90 μmol/L	35.00 - 75.00
ΑΘΡΟΙΣΜΑΤΑ-ΛΟΓΟΙ		
Ολικά Λιπαρά Οξέα :	3297.0 μmol/L	5950.0 - 11600.0
Ολικά Κορεσμένα Λιπαρά Οξέα :	1772.5 μmol/L	
Ολικά Μονοακόρεστα Λιπαρά Οξέα :	547.1 μmol/L	
Ολικά Πολυακόρεστα Λιπαρά Οξέα :	977.3 μmol/L	
Ολικά ω3 :	75.7 μmol/L	
Ολικά ω6 :	903.6 μmol/L	
ω6 / ω3 :	11.9	

15. Biochemical Tests Baseline (Participant 15I) Vitamin D



Dr. Dimitris Tsoukalas, MD
Chronic Diseases & Metabolic Disorders Clinic
Harvard Medical School Course in Gen. Internal Medicine Graduate
President of The European Institute of Nutritional Medicine
American College for the Advancement in Medicine

Δρ. Α. Μυλωνά, Δρ Ε. Παραμέρα, Δρ Γ. Ζαχαριουδάκης, Δ. Κατακουζηνός, Κ. Δουλφή

Όνοματεπώνυμο : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ

Ημ/νία Εξέτασης : 02/12/2016

ΒΙΟΧΗΜΙΚΟΙ ΜΕΤΑΒΟΛΙΤΕΣ ΣΤΟ ΠΛΑΣΜΑ - ΟΡΟ

Εξέταση	Αποτέλεσμα	Φυσιολογικές Τιμές
Βιταμίνη D (25-OH) :	↓ 48.5 ng/mL	50.0 - 80.0

16. Biochemical Tests Baseline (Participant 15I) Organic Acids

Διαγνωστική Ομάδα :

Παπακωνσταντίνου, Δρ. Α. Μυλωνά, Δρ Ε. Παραμέρα, Δ. Κατακουζηνός, Κ. Δουλή

15I






Εξεταζόμενος : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ

Ημ/νία : 02/12/2016

Ηλικία : 6 ετών

Φύλο : Άρρεν

ΜΕΤΑΒΟΛΟΜΙΚΟ ΠΡΟΦΙΛ ΑΝΑΛΥΣΗ ΟΡΓΑΝΙΚΩΝ ΟΞΕΩΝ

Εξέταση	Αποτ. - M.M	Φυσιολογικές Τιμές
Krebs Cycle		
Citric	63.5 mmol/mol Crea	0.0 - 656.0 
Aconitic	30.7 mmol/mol Crea	20.5 - 135.0 
Isocitric	5.3 mmol/mol Crea	16.0 - 99.0
2-ketoglutaric	18.8 mmol/mol Crea	41.0 - 82.0
Succinic	4.3 mmol/mol Crea	29.0 - 87.0
Fumaric	mmol/mol Crea	0.0 - 3.7
Malic	mmol/mol Crea	0.0 - 5.5
3-Hydroxy 3-methylglutaric	2.9 mmol/mol Crea	1.0 - 28.0 
Carbohydrate Metabolism		
Lactic	2.2 mmol/mol Crea	20.0 - 101.0
Pyruvic	3.6 mmol/mol Crea	3.5 - 22.0 
3-Hydroxybutyric	mmol/mol Crea	0.0 - 1.0
2-Ketoisovaleric	mmol/mol Crea	0.0 - 0.0
2-Hydroxyisocaproic	mmol/mol Crea	0.0 - 0.0
Pyroglutamic	10.1 mmol/mol Crea	0.0 - 61.0 
B-Complex Vitamin markers B1, B2, B3, B6,		
2-Ketoisovaleric	mmol/mol Crea	0.0 - 0.0
2-Ketoisocaproic	mmol/mol Crea	0.0 - 0.0
2-Keto 3-methylvaleric	mmol/mol Crea	0.0 - 0.0
3-Hydroxyisovaleric	15.7 mmol/mol Crea	0.0 - 10.0
Methylation Cofactor marker		
Methylmalonic	mmol/mol Crea	0.0 - 1.0

Διαγνωστική Ομάδα :
Παπακωνσταντίνου, Δρ. Α. Μυλωνά, Δρ Ε. Παραμέρα, Δ. Κατακουζηνός, Κ. Δουλφής

Εξεταζόμενος : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ

Ημ/νία : 02/12/2016

Ηλικία : 6 ετών

Φύλο : Άρρεν

ΜΕΤΑΒΟΛΟΜΙΚΟ ΠΡΟΦΙΛ ΑΝΑΛΥΣΗ ΟΡΓΑΝΙΚΩΝ ΟΞΕΩΝ

Εξέταση	Αποτ. - M.M	Φυσιολογικές Τιμές	
Neurotransmitter Metabolism markers (Tyrosine, Tryptophan, antioxidants)			
Homovanillic	2.7 mmol/mol Crea	0.7 - 10.3	20.83%
5-Hydroxyindoleacetic	1.1 mmol/mol Crea	0.0 - 8.7	12.64%
Vanillilmandelic	2.1 mmol/mol Crea	1.0 - 15.0	12.64%
Detoxification Indicators Arg, NAC, Met and antioxidants			
4-Hydroxyphenylacetic	9.4 mmol/mol Crea	0.0 - 7.0	
Orotic	mmol/mol Crea	0.0 - 1.9	
Glutaric	mmol/mol Crea	0.0 - 3.8	
2-Hydroxyglutaric	3.7 mmol/mol Crea	0.0 - 15.0	0.00%
Oxalate Metabolites			
Glycolic	32.0 mmol/mol Crea	43.0 - 172.0	
Oxalic	8.9 mmol/mol Crea	0.0 - 17.0	52.35%
Glyceric	mmol/mol Crea	0.0 - 2.0	
Ketone & Fatty Acid Oxidation			
2-Hydroxyisobutyric	4.3 mmol/mol Crea	0.0 - 5.0	86.00%
2-Hydroxybutyric	mmol/mol Crea	0.0 - 3.0	
Ethylmalonic	1.1 mmol/mol Crea	0.0 - 8.4	13.10%
Methylsuccinic	mmol/mol Crea	0.0 - 4.4	
Adipic	mmol/mol Crea	0.0 - 5.3	
Suberic	mmol/mol Crea	0.0 - 8.8	
Sebasic	mmol/mol Crea	0.0 - 1.5	
Q10			
3-Hydroxy 3-methylglutaric	2.9 mmol/mol Crea	1.0 - 28.0	7.04%
Succinic	4.3 mmol/mol Crea	29.0 - 87.0	
Methylcitric	mmol/mol Crea	0.2 - 5.8	

Διαγνωστική Ομάδα :

Παπακωνσταντίνου, Δρ. Α. Μυλωνά, Δρ Ε. Παραμέρα, Δ. Κατακουζηνός, Κ. Δουλφής






Εξεταζόμενος : ΠΑΠΑΔΟΠΟΥΛΟΣ ΚΩΝ/ΝΟΣ

Ημ/νία : 02/12/2016

Ηλικία : 6 ετών

Φύλο : Άρρεν

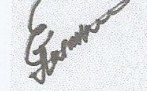
ΜΕΤΑΒΟΛΟΜΙΚΟ ΠΡΟΦΙΛ ΑΝΑΛΥΣΗ ΟΡΓΑΝΙΚΩΝ ΟΞΕΩΝ

Εξέταση	Αποτ. - Μ.Μ	Φυσιολογικές Τιμές
Biotin		
Methylcitric	mmol/mol Crea	0.2 - 5.8
Vitamin C		
Adipic	mmol/mol Crea	0.0 - 5.3
4-Hydroxyphenylpyruvic	mmol/mol Crea	0.0 - 6.0
Citric	63.5 mmol/mol Crea	0.0 - 656.0 
Antioxidant Status - Oxidative Damage		
Aconitic	30.7 mmol/mol Crea	20.5 - 135.0 
Citric	63.5 mmol/mol Crea	0.0 - 656.0 
Pyroglutamic	10.1 mmol/mol Crea	0.0 - 61.0 
Vitamin E and β-Carotene		
Adipic	mmol/mol Crea	0.0 - 5.3
2-Hydroxybutyric	mmol/mol Crea	0.0 - 3.0
3-Hydroxy 3-methylglutaric	2.9 mmol/mol Crea	1.0 - 28.0 

Σημειώσεις

Τα αποτελέσματα εκφράζονται σε mmol/mol Creatinine στα ούρα. Στην περίπτωση που δεν αναφέρεται συγκέντρωση μεταβολίτη, αυτή είναι κάτω από το όριο ανίχνευσης (1 mmol/mol Creatinine).

Ο Υπεύθυνος Εργαστηριακός



17. Participant Evaluation Questionnaire Intervention group (15I)

1

Αξιολόγηση Ασθενών (Ομάδα Παρέμβασης)

Εκ μέρους της ερευνητικής ομάδας θα θέλαμε να σας ευχαριστήσουμε που συμμετείχατε στη μελέτη αυτή. Θα εκτιμούσαμε αν αφιερώσετε λίγα λεπτά για την αξιολόγηση της. Η γνώμη σας θα μας βοηθήσει να παρέχουμε καλύτερες υπηρεσίες στους ασθενείς μας σε μελλοντικές μελέτες.

A. Αξιολόγηση της μελέτης

Παρακαλώ, βάλτε «X» στην απάντηση που σας ταιριάζει.

EA1. Η μορφή του ερωτηματολογίου ήταν εύκολα κατανοητή;

☒ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA2. Δόθηκε υποστήριξη από την ερευνητική ομάδα;

☒ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA3. Έχετε κάνει αλλαγές στην διατροφή του παιδιού κατά τη διάρκεια της παρέμβασης σε σύγκριση με τις διατροφικές συνήθειες της οικογένειας πριν την έναρξη της παρέμβασης;

☒ Ναι ☐ Όχι

Εάν ναι, ποιες αλλαγές έχετε κάνει;

Τρώει περισσότερα γαρί

.....

EA4. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την κατάσταση υγείας και του άσθματος στο παιδί σας;

☒ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς; Δεν αρρώσταζε συχνά όσο διάστημα ακολουθούσε το πρόγραμμα

EA5. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την ποιότητα ζωής του παιδιού σας (π.χ συμμετέχει καλύτερα στις καθημερινές δραστηριότητες-άθλημα, παιχνίδι, μελέτη κλπ.);

☒ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς;

EA6. Πώς ήταν η στάση του παιδιού σχετικά με την κατανάλωση ψαριών κατά τη διάρκεια της περιόδου των 6 μηνών;

☐ Θετική ☒ Αρνητική ☐ Αδιάφορη

EA7. Η στάση του παιδιού σας ήταν εμπόδιο για την τακτική κατανάλωση ψαριών;

☐ Ποτέ ☐ Σπάνια ☒ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ8. Υπήρχε ποτέ πρόβλημα στην αγορά λιπαρών ψαριών λόγω διαθεσιμότητας ή κόστους;

☒ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ9. Η προετοιμασία του γεύματος με ψάρι ήταν πρόβλημα λόγω έλλειψης χρόνου;

☒ Ποτέ ☐ Σπάνια ☐ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ10. Αντιμετωπίσατε κανένα πρόβλημα κατά τη διάρκεια της παρέμβασης; ☐ Ναι ☒ Όχι

Εάν ναι, πώς;

ΕΑ11. Πιστεύετε ότι αυτή η διατροφική παρέμβαση ήταν δύσκολο να εφαρμοστεί στην καθημερινή οικογενειακή ζωή σας;

☐ Ποτέ ☐ Σπάνια ☒ Μερικές φορές ☐ Τις περισσότερες φορές

ΕΑ12. Τώρα που έχει ολοκληρωθεί η παρέμβαση, σκοπεύετε να διατηρήσετε 2 γεύματα ψαριών την εβδομάδα ως μέρος του οικογενειακού σας μενού;

☒ Ναι ☐ Όχι

Αν όχι, γιατί;

ΕΑ13. Θα προτιμούσατε να δίνετε στο παιδί σας ένα συμπλήρωμα ωμέγα 3 ημερησίως αντί για 2 γεύματά λιπαρών ψαριών την εβδομάδα;

☒ Ναι ☐ Όχι ☐ Δεν ξέρω

Β. Μελλοντικές μελέτες

ΕΒ1. Θα σας ενδιέφερε να συμμετάσχετε σε άλλες διαιτητικές παρεμβάσεις στο μέλλον;

☐ Ναι ☐ Όχι ☒ Μπορεί

ΕΒ2. Πιστεύετε ότι μπορούμε να βελτιώσουμε αυτή την παρέμβαση;

☐ Ναι ☒ Όχι

Εάν ναι, προτείνετε πώς:

.....
.....

Σας Ευχαριστούμε!!!!!!!

17. Participant Evaluation Control Group (5C)

Παπαγεωργίου Αθηνών
5C 11/12/2017

Αξιολόγηση Ασθενών (Ομάδα Ελέγχου)

Εκ μέρους της ερευνητικής ομάδας θα θέλαμε να σας ευχαριστήσουμε που συμμετείχατε στη μελέτη αυτή. Θα εκτιμούσαμε αν αφιερώσετε λίγα λεπτά για την αξιολόγηση της. Η γνώμη σας θα μας βοηθήσει να παρέχουμε καλύτερες υπηρεσίες στους ασθενείς μας σε μελλοντικές μελέτες.

A. Αξιολόγηση της μελέτης

Παρακαλώ, βάλτε «X» στην απάντηση που σας ταιριάζει.

EA1. Η μορφή του ερωτηματολογίου ήταν εύκολα κατανοητή;

☒ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA2. Δόθηκε υποστήριξη από την ερευνητική ομάδα;

☒ Συμφωνώ απολύτως ☐ Συμφωνώ ☐ Διαφωνώ ☐ Διαφωνώ απόλυτα

EA3. Έχετε κάνει αλλαγές στην διατροφή του παιδιού κατά τη διάρκεια της παρέμβασης σε σύγκριση με τις διατροφικές συνήθειες της οικογένειας πριν την έναρξη της παρέμβασης;

☐ Ναι ☒ Όχι

Εάν ναι, ποιες αλλαγές έχετε κάνει;

.....

EA4. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την κατάσταση υγείας και του άσθματος στο παιδί σας;

☒ Ναι ☐ Όχι ☐ Δεν ξέρω

Εάν ναι, πώς; Μας δώσατε πολλή υγιεινή διατροφή για την καλύτερη διατροφή του παιδιού μας.

EA5. Πιστεύετε ότι αυτή η παρέμβαση βελτίωσε την ποιότητα ζωής του παιδιού σας (π.χ συμμετέχει καλύτερα στις καθημερινές δραστηριότητες-άθλημα, παιχνίδι, μελέτη κλπ.);

☐ Ναι ☐ Όχι ☒ Δεν ξέρω

Εάν ναι, πώς; Δεν ξέρω γιατί ήταν και πριν πολύ υγιεινή.

B. Μελλοντικές μελέτες

EB1. Θα σας ενδιέφερε να συμμετάσχετε σε άλλες διαιτητικές παρεμβάσεις στο μέλλον;

☒ Ναι ☐ Όχι ☐ Μπορεί

EB2. Πιστεύετε ότι μπορούμε να βελτιώσουμε αυτή την παρέμβαση; ☒ Ναι ☐ Όχι

Εάν ναι, προτείνετε πώς:

Κόπιας περισσότερη έρευνα για την αντιμετώπιση και ζωή του άσθματος.

Σας Ευχαριστούμε!!!!!!!

18.English Translations (Participant 15I)

Date: ...23.../...11.../2016.....

Screening Questionnaire

Completed by: interviewer

Refers to: Children 5-12 years

Respondents: Parents/Carers

Respondent's Details:

Name: [REDACTED] (mother)

SCREENING CRITERIA**Ep.6. How old is your child? (5-12 years old): .03.../.09.../.2010...**Was your child born between **2004** and **2011**? YES ☒ NO ☐**Ep.7. Does your child have mild-intermittent asthma?** YES ☒ NO ☐If the answer to the above two questions is YES, then continue with the **EXCLUSION CRITERIA**.However, if the answer to at least one of the two questions is NO, then the child does not qualify to participate in this study.**EXCLUSION CRITERIA****Ep.8.** Perhaps your child does not eat the following fatty fish: sardines, salmon, trout, anchovies, gilthead sea bream, chubb mackerel, mackerel?YES, he does not eat ☐ NO, he eats ☒

If he does not eat, why?

He is allergic ☐ He/she does not like to eat this kind of fish ☐ He/she is vegetarian ☐**Ep.9. Does your child suffer from any of the following medical conditions?**GERD YES ☐ NO ☒Cystic Fibrosis YES ☐ NO ☒Congenial Pulmonary airway disease YES ☐ NO ☒**Ep.10. Does your child take fish oil supplements?**YES ☐ NO ☒

If the answer to at least one of the above questions of the exclusion criteria (Q3-Q5) is YES, then your child is **not eligible to participate in this study**. On the other hand, if the response to ALL of the questions of the exclusion criteria is **NO**, then your child is **ELIGIBLE** to participate. If the parent/carer of the eligible child agrees and signs the consent form, then the child is becomes a **PARTICIPANT** in this study and will be assigned a participant identification code (ID).



School of Allied Health
Department of Rehabilitation, Nutrition & Sport
Melbourne, 3086, Australia

Participant ID: 15I.....

PARTICIPANT CONSENT FORM

I, *Adamantia Papadopoulou*, as the parent/guardian of my child *Constantinos Papadopoulos*,
I give my child the permission, provided that he/she agrees to participate in the study “The prophylactic potential of a Mediterranean dietary pattern enriched with oily fish in asthmatic children” conducted by La Trobe University, Melbourne, Australia.

I declare that I am the person responsible for giving my child permission *Constantinos* to consent in participating in this study.

I understand that the study involves the completion of a questionnaire and consumption of two fish meals per week over a period of 6 months. I have read or have had read to me and understood the **participant information statement and consent form**, and any questions that I have asked or my child have been answered to our satisfaction. I understand that even though we agree to be involved in this project, he/she can withdraw from the study at any time, and we can withdraw our data up to four weeks following the completion of our participation in the research. Further, in withdrawing from the study, I can request that no information from our involvement be used. I agree that research data provided by us or with our permission during the project may be included in a PhD thesis, presented at conferences and published in journals on the condition that neither our names nor any other identifying information is used.”

Name of Participant (block letters): *Adamantia Papadopoulou*

Signature:*Signed by parent*.....

Date: 23/11/2016

Participating Doctor & Supervisor: Dr. Ch. Katsardis

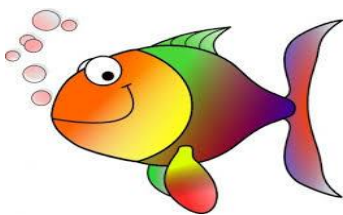
Investigator: Maria. M. Papamichael

Student Supervisor: Dr.C. Itsiopoulos

Signature:

Signature:

Signature:



IDENTIFICATION AND CONTACT DETAILS

Date: ...23... /11/ 2016....

Participant's ID: 15I

Socio-demographic Questionnaire

Completed by: parent/guardian

Refers to: Children 5-12 years

Respondents: Parents/ or guardians

Details of Parents/or guardian completing this questionnaire

Name of respondent: [REDACTED] (mother)

Q.1. What is your relationship with the child?

Mother ☒ Grandfather ☐
 Father ☐ Grandmother ☐
 Guardian ☐

Q.2. What nationality are you?

☒ Greek Other ☐

Q.3. What race do you belong to?

Caucasian ☒ Black/or African ☐ Asian ☐

Q.4. What is your marital status? Are you.....

Single ☐
 Married ☒
 Widowed ☐
 Divorced ☐
 Separated ☐
 Living together ☐

Q.5. What is your current employment status? Are you.....

Unemployed

☐

Working Full-time

☐

Working Part-time

☐

Self-Employed

☐

Working in Public Sector

☐

Working in Private Sector

☒

Retired

☐

Put off work or closed business

☐

House-wife or baby sitter

☐

Unable to work or permanently handicapped

☐

Other case unable to work

☐

Q.6. What education level have you completed?

Primary School	<input type="checkbox"/>	Technical College	<input type="checkbox"/>
Junior High	<input type="checkbox"/>	University	<input type="checkbox"/>
Senior High	<input type="checkbox"/>	Masters	<input type="checkbox"/>
College	<input checked="" type="checkbox"/>	PhD	<input type="checkbox"/>

Details of spouse**Q.7. What nationality is your spouse?**

Greek ☒ Other ☐

Q.8. What race does your spouse belong to?

Caucasian ☒ Black/or African ☐ Asian ☐

Q.9. What is your spouse current employment status?

Unemployed	<input type="checkbox"/>
Working Full-time	<input type="checkbox"/>
Working Part-time	<input type="checkbox"/>
Self-Employed	<input type="checkbox"/>
Working in Public Sector	<input type="checkbox"/>
Working in Private Sector	<input checked="" type="checkbox"/>

- Retired ☐
- Put off work or closed business ☐
- House-wife or baby sitter ☐
- Unable to work or permanently handicapped ☐
- Other case unable to work ☐

Q.10. What education level has your spouse completed?

- | | |
|---|--|
| Primary School <input type="checkbox"/> | Technical College <input type="checkbox"/> |
| Junior High <input type="checkbox"/> | University <input type="checkbox"/> |
| Senior High <input type="checkbox"/> | Masters <input type="checkbox"/> |
| College <input checked="" type="checkbox"/> | PhD <input type="checkbox"/> |

Q.11. What is your family's monthly income? (include total income not only due to employment and rent)

.....700 €.....

Child's Details

Q. 12. How many children are in the family?

Number of children in the family 2

Q .13. What ranking is the participating child in your family?

First ☐ Second ☒ Third ☐ Fourth ☐

Q 14. What sex is the participating child? ☒ Boy ☐ Girl

Q 15. What is the participant's date of birth? *03/ 09 / 2010*

Q 16. What weight and height is your child today? *24.5 kg* *123 cm*

Q 17. What type of school does the participant attend? Private ☐ ☒ Public

Asthma Control Questionnaire

This questionnaire is to be completed by the child together with the parent/guardian.
Think how the child's asthma was **during the PAST WEEK**, and mark with a **circle** the response which best describes your condition.

Q.1. During the past week, how often were you woken by your asthma during the night?

- ☒ 0 Never
- 1 Hardly ever
- 2 A few times
- 3 Several times
- 4 Many times
- 5 A great many times
- 6 Unable to sleep because of asthma

Q.2. During the past week, how bad were your symptoms (e.g hard to breathe, wheeze, cough) when you woke up in the morning?

- ☒ 0 No symptoms
- 1 Very mild symptoms
- 2 Mild symptoms
- 3 Moderate symptoms
- 4 Quite severe symptoms
- 5 Severe symptoms
- 6 Very severe symptoms

Q.3. During the past week, how limited were you in your activities because of your asthma (e.g absent from school or lessons)?

- ☒ 0 Not limited at all
- 1 Very slightly limited
- 2 Slightly limited
- 3 Moderately limited
- 4 Very limited
- 5 Extremely limited
- 6 Totally limited

Q4. During the past week, how much shortness of breathe did you experience because of your asthma?

- ☐ 0 None
- ☐ 1 A very little
- ☐ 2 A little
- ☐ 3 A moderate amount
- ☐ 4 Quite a lot
- ☐ 5 A great deal
- ☐ 6 A very great deal

Q5. During the past week, how much of the time did you wheeze?

- ☐ 0 Never
- ☐ 1 Hardly any of the time
- ☐ 2 A little of the time
- ☐ 3 A moderate amount of the time
- ☐ 4 A lot of the time
- ☐ 5 Most of the time
- ☐ 6 All of the time

Q6. During the past week, how many puff/inhalations of your reliever have you used each day? (e.g Aerolin / Serevent)?

(If you are not sure how to answer this question please ask for assistance)

FEV1% Pred 102%
(well-controlled)

- ☐ 0 None
- ☐ 1 1-2 puffs/inhalations most days
- ☐ 1 3-4 puffs/inhalations most days
- ☐ 3 5-8 puffs/inhalations most days
- ☐ 4 9-12 puffs/inhalations most days
- ☐ 5 13-16 puffs/inhalations most days
- ☐ 6 More than 16 puffs/inhalations most days

Q7. Since your last check up at the asthma clinic, did you have an unexpected visit to the hospital or to emergency admissions or to the asthma specialist?

YES ☐

NO ☒

IF YES, how many times

MINI PAEDIATRIC ASTHMA QUALITY OF LIFE QUESTIONNAIRE

Please complete all questions by circling the number that best describes how you have been during the last week as a result of your asthma.

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK BY:

	Extremely bothered	Very bothered	Quite bothered	Somewhat bothered	Bothered a bit	Hardly bothered at all	Not bothered
Q8. COUGHING	1	2	3	4	5	6	⑦
Q9. WHEEZING	1	2	3	4	5	6	⑦
Q10. TIGHTNESS IN CHEST	1	2	3	4	5	6	⑦

IN GENERAL, HOW OFTEN DURING THE LAST WEEK DID YOU:

Q11. Feel OUT OF BREATHE?	1	2	3	4	5	6	⑦
Q12. Feel TIRED because of your asthma?	1	2	3	4	5	6	⑦
Q13. Have trouble sleeping AT NIGHT because of your asthma?	1	2	3	4	5	6	⑦
Q14. Feel FRUSTRATED because of your asthma?	1	2	3	4	5	6	⑦
Q14. Feel FRIGHTENED OR WORRIED because of your asthma?	1	2	3	4	5	6	⑦
Q16. Feel IRRITABLE (cranky/grouchy) because of your asthma?	1	2	3	4	5	6	⑦
Q17. Feel DIFFERENT or LEFT OUT because of your asthma?	1	2	3	4	5	6	⑦

HOW BOTHERED HAVE YOU BEEN DURING THE LAST WEEK DOING?

Q18. PHYSICAL ACTIVITIES (such as running, swimming, uphill/upstairs and cycling)?	1	2	3	4	5	6	⑦
Q19. BEING WITH ANIMALS (such as playing with pets and looking after animals)?	1	2	3	4	5	6	⑦
Q20. ACTIVITIES WITH FRIENDS AND FAMILY (such as playing at recess and doing things with your friends and family)?	1	2	3	4	5	6	⑦

Q21. How many times during the week does your child exercise?

Never/rarely ☐ 1-2 times/week ☐ More than 3 times/week ☒

If YES, what sport does your child participate in/or sports (e.g soccer, basketball, swimming)? *Swimming, Tae Kwon Do, gymnastics, soccer*

Q22. For how long usually does your child play sport?1-2.hours/ day, 6..times/week
If NOT, why?

Does he/she have an asthma attack when he/she plays sport? ☐ Other ☐

Dietary Habits Questionnaire

Q.1. Does your child eat fast food more than once a week (e.g Goody's or fast-food restaurants) ?

YES ☐ NO ☒

Q.2. How many times per week does your child eat breakfast?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☒

Q.3. If your child eats breakfast, does he/she eat one of the following foods and how many times per week?

Breakfast cereal or bread or rusks or toast NO ☐ YES ☐ times/ week.....

Milk products (milk, yogurt, cheese) NO ☐ YES ☒ times/week...7....

Cheese-pie or croissant or cookies or cake NO ☐ YES ☒ times/week 1.....

If NO, what does your child usually eat for breakfast and how many times per week?

Q.4. Do you use olive oil for cooking, frying, or add to pasta and rice?

YES ☒ NO ☐ If NO, what do you use?

Instructions:

For every food item, indicate the number of times and amount that your child consumes.

For example, if your child drinks **1 cup** of milk **two times per day**, mark “**1**” in the category “**2-3 times/day**”.

If your child eats **2 fruits once a day**, mark “**2**” in the category ‘**once/day**’.

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Milk (1 Cup)							1	
Fruit (1 medium or ½ C)						2		

(1 Cup = 1 Tea Cup (240ml); 1 Tblsp= 1 tablespoon or soup spoon; 1 teas= 1 teaspoon, 1 item= 1 piece; 1 tub=1 tub of yogurt (200 g))

How many times and serves does your child eat/drink.....?

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Q.5. Milk (1 cup.)						1		
Q.6. Chocolate milk (1 cup.)	X							
Q.7. Yogurt (1 tub)					1			
Q.8. Cheese (white or yellow) (e.g feta or kasseri) (40g)				1				
Q.9. Fruit (1 medium)						1		
Q.10. Fruit Juice (1 cup)				1				
Q.11. Salads Raw (e.g , cabbage, rocket, carrots, tomato, lettuce, cucumber) (1/2-1 cup)				1				
Q.12. Vegetables Boiled (e.g broccoli, cabbage, cauliflower, collard greens, green beans, marrows, silverbeet, spinach, beetroot) (1/2-1 cup)	X							
Q.13. Stewed vegetables in sauce e.g (Lady fingers, biam, green beans) (1/2- 1 cup.)	X							
Q.14. Legumes (1 plate -300 g)			1					
Q.15. Cereals e.g [Breakfast cereals (1/2 cup), Bread (1 slice=30 g), Rusks (2)]						1		
Q.16. Pasta (1 cup cooked 140 g)			1					
Q.17. Rice (1 cup cooked 160 g)				1				
Q.18. Red Meat e.g [Pork/Lamb/ Beef / Goat/Rissoles/ Bifteki (2 items)] (150 g cooked= restaurant serve)				1				
Q.19. White Meat e.g [Chicken/ Rabbit/ Turkey] (150 g)			1					
Q.20. Pastitsio/ Spaghetti Moussaka / Bolognaise/ Papoutsakia/ Stuffed vegetables with rice (150 g restaurant serve)			1					

Food (serving size)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	4 or more times/ day
Q.21.Seafood e.g [Calamari, Cuttlefish, Prawns, Mussels,Octopus] (150 g = restaurant serve)	X							
Q.22. Lean Fish e.g [Pandora, Whiting, Garfish, Smelt, John Dory, White Bait, Cod, Hake, Flounder, Bogue, Swordfish, Tuna Red Mullet] (150 g = restaurant serve)			1					
Q.23.Fatty Fish e.g [Sardines/anchovies (12 pieces), Salmon, Trout, Mackerel, Chubb Mackerel, Gilthead Seabream] (150 g)			1					
Q.24.Margarine (1 teas)				1				
Q.25.Nuts (1 handful/ 1/3 cup/ 50 g)	X							
Q.26.Olive Oil (1Tblesp)						1		
Q.27.Fast Food e.g [Hamburger (1 item), Souvlaki with pita (1), Pizza (2 pieces) Hot Dog (1)]		1						
Q.28. Pies e.g [Cheese pies, Spinach pies] (1 serve = 150g)		1						
Q.29.Sweets e.g [Cream cakes (1item) Cookies (2),Biscuits (2), Cakes (1 piece), Croissant (1), Ice-cream (1 ball), Milk-shake (1 cup), Chocolate (60g)]					1			
Q.30.Salty snacks [e.g potato chips, twistees, popcorn](1 packet 70g)				1				
Q.31. Soft drinks/ Energy drinks (1 can 330 ml)		1						

Thank you very much

Medical History Questionnaire

Mother's Details

Q.1. Did you smoke during pregnancy? YES ☒ NO ☐

Q.2. Did you smoke during the first year of your child's life? ☒ YES NO ☐

Q.3. Do you currently smoke? YES ☒ NO ☐

If YES, what brand of cigarettes?
.....

If YES, how many cigarettes per day ☐ or packets of cigarettes per day ☐?

Q.4. During childhood (0-16 years old), did you suffer from? YES ☐ NO ☒

Asthma? ☐

Rhinitis (Hay fever)? ☐

Eczema? ☐

Q.5. During adulthood (from 16 years onwards till today) do you suffer from ...?

YES ☐ NO ☒

Asthma? ☐

Rhinitis (Hay fever)? ☐

Eczema? ☐

Father's Details

Q.6. Did the father smoke during the first year of the child's life? YES ☐ NO ☒

Q.7. Does the father smoke today? YES ☐ NO ☒

If YES, what brand of cigarettes?
.....

If YES, how many cigarettes per day ☐ or packets of cigarettes per day ☐

Q.8. During childhood (0-16 years), did the father suffer from.... YES ☐ NO ☒

Asthma? ☐

Rhinitis (Hay fever)? ☐

Eczema? ☐

Q.9. During adulthood (from 16 years old till today) did the father suffer from...?

YES ☐ ☒ NO

Asthma? ☐ Rhinitis ? ☐ Eczema? ☐

The following questions refer to pregnancy and lactation details and the child's medical history

Participant's Details

Q.10. How many weeks was the term of your pregnancy?

32-39 weeks ☐ 37-40 weeks ☒ >40 weeks ☐

Q.11. How much did your child weigh at birth?

< 2500 grams ☐ 2500- 4000 grams ☒ >4000 grams ☐

Q.12. Was your child born by.....?

Vaginal delivery ☐ Caesarean-section ☒

Q.13. Did you breast-feed your child? YES ☒ NO

If YES, for how long? <3 months ☐ 3-6 months ☐ 6-12 months ☒ >12 months ☐

Q.14. At what age was your child diagnosed with asthma? 4 months

Q.15. Does your child suffer from any other allergies? YES ☒ NO ☐

If, YES, does he suffer from ?

Rhinitis/or Hay fever? ☒ Conjunctivitis? ☐ Eczema ☒

Food Allergy ☐ If, YES, what?NO.....

Q.16. During the last month has your child taken medication YES ☒ NO ☐




If YES, when.....?

As part of a daily therapy ☒ For how long? *Nov- May*weeks





Only as needed during episodes ☐ How many episodes did he/she have?times






When he/she is sick ☐ How many times was he/she sick? times

Q17. What asthma medication does your child take and dosage?

MEDICATION TYPE			DOSAGE
Bronchodilators			
Aerolin (100 µg)		<input type="checkbox"/>times/day
Anti-inflammatory corticosteroids			
Flixotide (125 µg)		<input type="checkbox"/>times/day
Flixotide (250 µg)		<input type="checkbox"/>times/day

MEDICATION TYPE			DOSAGE
Seretide (125 µg)		<input type="checkbox"/>times/day
Seretide (250 µg)		<input type="checkbox"/>times/day
Seretide (Discus) (100 µg)		<input type="checkbox"/>times/day
Seretide (Discus) (250 µg)		<input type="checkbox"/>times/day
Symbicort (80 µg)		<input type="checkbox"/>times/day
Symbicort (160 µg)		<input type="checkbox"/>times/day
Anti-Leukotrienes			

MEDICATION TYPE			DOSAGE
Singulair (Montelukast) (5 mg)		<input checked="" type="checkbox"/> 1..times/day
Miralust (Montelukast)(5 mg)		<input type="checkbox"/>times/day
Apilone (Montelukast) (5 mg)		<input type="checkbox"/>times/day
Modulair (Montelukast) (5 mg)		<input type="checkbox"/>times/day
Cortisone (per os)			
Medrol per os (16 mg) (Prednisolone)		<input type="checkbox"/>times/day
Prezolon (5 mg)		<input type="checkbox"/>times/day
RHINITIS			
Soldesanil drops		<input type="checkbox"/>times/day

MEDICATION TYPE		DOSAGE	
Nasonex		<input type="checkbox"/>times/day
Mometasone nasal spray		<input type="checkbox"/>times/day
Pulmicort nasal		<input type="checkbox"/>times/day
ANTI-HISTAMINES			
AERIUS (syrup) AERIUS (tablets)		<input type="checkbox"/>times/day
XYZAL (syrup) XYZAL (tablets)		<input type="checkbox"/>times/day

Q18. Does your child takeNutritional supplements ☐Vitamins ☐

None of the two

☒ X

If YES, which one?

How many tablets per day?

Participant ID: 15I

KIDMED Test

Date: 26/11/2016

Does your child

Q1. Take a fruit or fruit juice every day? YES ☒ NO ☐Q2. Eat two fruits every day? YES ☒ NO ☐Q3. Eat fresh salad or cooked vegetables regularly once a day? YES ☐ NO ☒Q4. Eat fresh salad or cooked vegetables more than once a day? YES ☐ NO ☒Q5. Eat fish regularly (at least 2-3 times per week)? YES ☐ NO ☒

If YES, how much fish does the participant eat per meal?

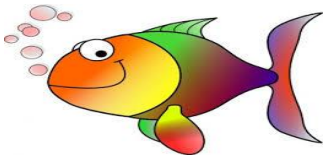
60-90g * ☐ 90-120 g ☒ 120-150 g ☐ More than 150 g ☐

What type of fish usually eats? e.g gilthead seabream, sardines, whiting

*Salmon,, gilthead seabream*Q6. Go to a fast-food restaurant (hamburger) more than once a week? YES ☐ NO ☒Q7. Eats legumes more than once a week? YES ☐ NO ☒

Q8. Eats pasta or rice almost every day (5 or more times per week)?

YES ☐ NO ☒Q9. Eats cereals or grains (bread etc.) for breakfast? YES ☐ NO ☒Q10. Eat dairy products for breakfast (yogurt, milk, cheese etc.)? YES ☒ NO ☐Q11. Eat baked goods or pastries (e.g pies, cookies, croissant) for breakfast? YES ☐ NO ☒Q12. Skips breakfast? YES ☐ NO ☒Q13. Eat nuts regularly (at least 2-3 times per week)? YES ☐ NO ☒Q14. Eat 2 yogurts and/or some cheese (40 g) daily? YES ☒ NO ☐Q15. Eat sweets and candy several times every day? YES ☒ NO ☐Q16. Eat olive oil with meals? YES ☒ NO ☐



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HEALTH CLINICS FOR AUTOIMMUNE AND CHRONIC DISEASES



Βασιλόπουλος
...και του πουλιού το γάλα!

ΣΚΑΛΑΒΕΝΙΤΗΣ

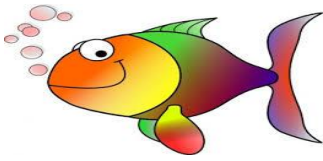
Date: ...26.../...11.../...2016....

Participant ID: 15 I /...../...../.....

24 Hr Dietary Recall 1 (baseline) (Saturday)

What did your child eat yesterday?

Meal	Food	Quantity	Cooking Method
Breakfast	1 C Milk full cream 4%	1 cup (250ml)	
Mid-Morning snack	1 Toast with ham/cheese	2 slices bread (60 g) 30 g ham 30 g cheese	
Lunch (at school)	Macaroni + cheese	Normal serve (300 g) 2 tblesp grated cheese	
Afternoon Snack	1 slices bread +- Merenda (chocolate hazelnut spread) + 1 fruit (banana)	60 g 1 tblesp	
Dinner	Milk 4% + Cornflakes with chocolate bits	1 cup (250 ml) 1 cup (250 ml)	
Snack	-		



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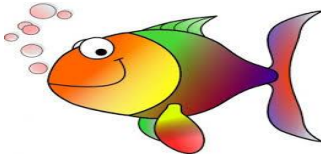
Date: ...17.../...2.../...2017....

Participant ID: 15 I /...../...../.....

24 Hr Dietary Recall 2 (3 months) (Friday)

What did your child eat yesterday?

Meal	Food	Quantity	Cooking Method
Breakfast	Milk 4%	1 cup	
Mid-Morning snack	1 chocolate croissant-	70g-	
Lunch	Mincemeat and rice ball 1 carrot	3 balls	(No veges) (No salads)
Afternoon Snack	Toasted sandwich+ Cheese (1 slice)+ turkey ham (1 slice) 1 juice (AMITA) 2 truffles	60 g slice bread 30 g 30 g 250 ml 2 small balls	
Dinner	1 pork souvlaki + 2 slices bread	120 g 60 g	
Snack	-		



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Βασιλόπουλος
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Date: ...25/6/2017.... (Follow-up)

Participant ID: 15 I //...../.....

24 Hr Dietary Recall 3 (Sunday)

What did your child eat yesterday?

Meal	Food	Quantity	Cooking Method
Breakfast	1 C Milk 4%	1 cup	
Mid-Morning snack	1 wafer biscuit (AMARETTI)	1 bar 70g	
Lunch	Pastitsio+ Tomato salad+ Bread+ Feta cheese	1 restaurant serve (300 g) 1 cup 30 g 60 g	Oven-cooked
Afternoon Snack	1 banana	100 g	
Dinner	Pastitsio	1 serve (restaurant) (300 g)	
Before sleep	Milk 4%	>300 g	

Record of weekly fatty fish intake

Participant's name (15 I)...Constantinos Papadopoulos.....

Instructions: Please record every week the 2 days that your child consumes fatty fish and the amount eaten at each meal.

For example, if your child consumes the first week, say Tuesday 150 g anchovies (15 fish) and on Saturday 200 g trout (weight cooked without head and bones), you will record in 'Week', day "Tuesday" 150 g anchovies and day "Saturday" 200 g trout (see example highlighted in green)

Starting date Consumption of fatty fish23/..11...../..2016.....	Record						
	Consumption of fatty fish						
	2 times /per week at least 150g cooked fatty fish per meal						
WEEK	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Example		150 g anchovies (15 fish)				200g trout	
WEEK 1						15 sardines	15 sardines
WEEK 2		150 g salmon		150 g salmon			
WEEK 3	150 g GH Sea bream				150g salmon		
WEEK 4		150 g GH Sea bream					150 g salmon
WEEK 5			15 sardines				150 g GH Sea bream
WEEK 6		150 g GH Sea bream				15 sardines	
WEEK 7			150 g salmon			150 g salmon	
WEEK 8		15 sardines				150 g salmon	
WEEK 9		150 g GH Sea bream				20 anchovies	
WEEK 10			150 g salmon				150 g salmon
WEEK 11			150 g GH Sea bream				150 g GH Sea bream
WEEK 12				150 g salmon			150 g GH Sea bream
WEEK 13			150 g GH Sea bream				150 g salmon
WEEK 14			150 g salmon				150 g GH Sea bream
WEEK 15			150 g GH Sea bream				150 g salmon
WEEK 16			150 g salmon				150 g salmon
WEEK 17				150 g GH Sea bream			150 g GH Sea bream
WEEK 18			15 sardines				15 sardines
WEEK 19			150 g GH Sea bream				150 g GH Sea bream
WEEK 20			15 sardines				150 g GH Sea bream
WEEK 21			15 sardines				15 sardines
WEEK 22		150 g salmon				150 g salmon	
WEEK 23		150 g salmon				150 g salmon	
WEEK 24		150 g salmon				15 sardines	
Date ending at 6 months17..../...05...../...17....							

Key: GS Sea bream- Gilthead Sea Bream

Participant: 15 I

Date: 17.5.17

Participant Evaluation (Intervention Group)

On behalf of the research group we would like to thank you for participating in this study. We would appreciate if you would take a few minutes to complete the study evaluation. Your opinion will help us provide a better service to our patients in future trials.

A. Study evaluation

Please mark with an "X" your response to the following questions:

QA1. The questionnaire format was easy to understand

☒ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA2. Support was provided by the research team at all times.

☒ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA3. Have you made any changes in the child's diet during the intervention (apart from the increase in fish intake) as compared to the family's dietary habits at the start of the intervention?

☒ Yes ☐ No

If yes, what changes have you made to the child's diet? *He eats more fish*

QA4. Do you believe that this intervention improved your child's health and asthma status?

☒ Yes ☐ No ☐ I don't know

If yes, indicate how? *He didn't get ill often while he was following the program*

QA5. Do you believe that this intervention improved your child's well-being and quality of life (able to engage better in daily activities-sport, play, study etc.)

☒ Yes ☐ No ☐ I don't know

If yes, indicate how

.....

QA6. How was the child's attitude regarding fish consumption during the 6 month period?

☐ Positive ☒ Negative ☐ Indifferent

QA7. Was the child's attitude a barrier for regular fish consumption?

☐ Never ☐ Occasionally ☒ Sometimes ☐ Most of the time

QA8. At any time was there a problem in purchasing fatty fish due to availability or cost?

☒ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA9. Was the preparation of fish meals a problem due to lack of time?

☒ Never ☐ Occasionally ☐ Sometimes ☐ Most of the time

QA10. Did you encounter any problems during this intervention? Yes ☐ ☒ No

If yes, please indicate

.....

QA11. Do you feel that this dietary intervention was difficult to apply in your daily family life?

☐ Never ☐ Occasionally ☒ Sometimes ☐ Most of the time

QA12. Now that this intervention has ended, do you intend to maintain 2 fish meals per week as part of your family menu? ☒ Yes ☐ No

If no, why not?

.....

QA13. Would you have preferred to give your child an Omega 3 supplement daily as an alternative to fatty fish consumption twice weekly?

☒ Yes ☐ No ☐ I don't know

If yes, why?

.....

B. Future Studies

QB1. Would you be interested in taking part in other dietary interventions in the future?

☐ Yes ☐ No ☒ Maybe

QB2. Do you think that we can improve this intervention in anyway? ☐ Yes No ☒

If yes, please suggest how:

.....

Thank you!!!!!!!!!!

Participant Evaluation (Control group)

Participant: 5C

Date: 11.5.2017

On behalf of the research group we would like to thank you for participating in this study. We would appreciate if you would take a few minutes to complete the study evaluation. Your opinion will help us provide a better service to our patients in future trials.

A. Study evaluation

Please mark with an "X" your response to the following questions:

QA1. The questionnaire format was easy to understand

☒ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA2. Support was provided by the research team at all times

☒ Strongly agree ☐ Agree ☐ Disagree ☐ Strongly disagree

QA3. Have you made any changes in the child's diet during the intervention as compared to the family's dietary habits at the start of the intervention?

☐ Yes ☒ No

If yes, what changes have you made to the child's diet?

.....

QA4. Do you believe that this intervention improved your child's health and asthma status?

☒ Yes ☐ No ☐ I don't know

If yes, indicate how?

It gave us a lot of good information regarding better nutrition for our child.

.....

QA5. Do you believe that this intervention improved your child's well-being and quality of life (able to engage better in daily activities-sport, play, study etc.)

☐ Yes ☐ No I don't know ☒

If yes, indicate how ? *I don't know why, but he was well before*

B. Future Studies

QB1. Would you be interested in taking part in other dietary interventions in the future?

☒ Yes ☐ No ☐ Maybe

QB2. Do you think that we can improve this intervention in anyway? ☒ Yes ☐ No

If yes, please suggest how?

By doing more research on the management and prevention of asthma development.

Thank you!!!!!!!!!!!!

Appendix 2D Results: Dietary Assessment

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Children's frequency of consumption of food over the past month at baseline for the total sample is presented in Table 1 and at six-months in Table 2.

Table 1 Children's frequency of consumption of food items over the last month at baseline

FFQ Baseline	How many times in the last month did your child consume.....?							
Food item /Frequency of intake (%)	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	≥ 4 times/ day
Milk	1.6	-	3.1	6.3	10.9	43.8	32.8	1.6
Chocolate Milk	59.4	7.8	12.5	3.1	3.1	7.8	6.3	-
Yogurt	14.1	20.3	20.3	34.4	4.7	4.7	1.6	-
Cheese (white/yellow)	9.4	4.7	12.5	20.3	21.9	23.4	7.8	-
Fruit	3.1	4.7	3.1	23.4	14.1	43.8	7.8	-
Fruit juice	10.9	4.7	9.4	35.9	7.8	28.1	3.1	-
Stewed vegetables in sauce	12.5	12.5	45.3	29.7	-	-	-	-
Boiled vegetables	32.8	17.2	23.4	20.3	3.1	3.1	-	-
Salads	17.2	12.5	9.4	37.5	9.4	14.1	-	-
Legumes	3.1	7.8	56.3	32.8	-	-	-	-
Cereals	7.8	-	9.4	25.0	26.6	26.6	3.1	1.6
Pasta	1.6	3.1	51.6	34.4	7.8	-	1.6	-
Rice	10.9	25.0	46.9	15.6	-	-	1.6	-
Red Meat	4.7	-	29.7	59.4	4.7	1.6	-	-
White Meat	-	3.1	56.3	37.5	1.6	1.6	-	-
Traditional Meals (pastitsio, mousaka)	6.3	42.2	42.2	9.4	-	-	-	-
Seafood	59.4	29.7	9.4	1.6	-	-	-	-
Lean Fish	21.9	35.9	35.9	6.3	-	-	-	-
Fatty Fish	23.4	35.9	35.9	4.7	-	-	-	-
Margarine	59.4	9.4	6.3	15.6	6.3	3.1	-	-
Nuts	42.2	39.1	7.8	9.4	1.6	-	-	-
Olive oil	1.6	-	1.6	4.7	25.0	45.3	14.1	7.8
Fast Food	17.2	40.6	32.8	7.8	-	1.6	-	-
Pies	23.4	26.6	26.6	18.8	-	4.7	-	-
Sweets	3.1	10.9	9.4	42.2	23.4	10.9	-	-
Salty Snacks	21.9	28.1	25.0	23.4	1.6	-	-	-
Soft/Energy drinks	64.1	20.3	6.3	9.4	-	-	-	-

Table 2 Children's frequency of consumption of food items over the last month at six-months

FFQ Six-months Food item/Frequency of intake (%) N=64	How many times in the last month did your child consume.....?							
	Never/ rarely	1-3 times/ month	Once/ week	2-3 times/ week	4-6 times/ week	Once/ day	2-3 times/ day	≥ 4 times/ day
Milk (1 Cup)	1.6	-	-	12.5	12.5	35.9	35.9	1.6
Chocolate Milk (1 Cup)	62.5	15.6	6.3	-	4.7	4.7	6.3	-
Yogurt (200 g)	23.4	20.3	18.8	23.4	7.8	6.3	-	-
Cheese (white/yellow) (30 g)	6.3	6.3	6.3	28.1	28.1	20.3	4.7	-
Fruit (1 medium)	1.6	7.8	1.6	21.9	28.1	25.0	14.1	-
Fruit juice (1 glass)	3.1	9.4	9.4	42.2	7.8	25.0	3.1	-
Stewed vegetables in sauce	17.2	14.1	42.2	26.6	-	-	-	-
Boiled vegetables	35.9	17.2	29.7	15.6	-	1.6	-	-
Salads (1 Cup)	14.1	1.6	9.4	37.5	15.6	20.3	1.6	-
Legumes (300 g)	4.7	10.9	60.9	23.4	-	-	-	-
Cereals (30 g)	6.3	1.6	3.1	25.0	18.8	35.9	7.8	1.6
Pasta	9.4	-	45.3	42.2	3.1	-	-	-
Rice	10.9	21.9	50.0	14.1	3.1	-	-	-
Red Meat (150 g)	-	9.4	28.1	54.7	6.3	1.6	-	-
White Meat (150 g)	-	12.5	57.8	26.6	3.1	-	-	-
Traditional Meals (pastitsio, mousaka)	4.7	45.3	42.2	7.8	-	-	-	-
Seafood (150 g)	46.9	43.9	9.4	-	-	-	-	-
Lean Fish (150 g)	34.4	34.4	20.3	9.4	1.6	-	-	-
Fatty Fish (150 g)	17.2	23.4	23.4	34.4	1.6	-	-	-
Margarine (1 Tblsp.)	62.5	7.8	10.9	10.9	3.1	4.7	-	-
Nuts (50 g)	37.5	42.2	10.9	7.8	1.6	-	-	-
Olive oil (1 Tblsp.)	3.1	1.6	1.6	4.7	23.4	39.1	18.8	7.8
Fast Food (1 portion)	9.4	48.4	34.4	7.8	-	-	-	-
Pies (1 portion)	9.4	37.5	34.4	15.6	1.6	1.6	-	-
Sweets (60 g)	4.7	6.3	21.9	43.8	9.4	12.5	1.6	-
Salty Snacks (70 g)	10.9	43.8	31.3	7.8	3.1	3.1	-	-
Soft drinks/Energy drinks (330 ml)	51.6	17.2	14.1	15.6	-	1.6	-	-

In Tables1 and 2, analysis of FFQs at both time points showed that in general children had low consumption of milk products, fruit, vegetables, fish, nuts, cereals, rice/pasta and legumes. Specifically, about 30% of children drank milk 2-3cups/day, 20% one slice of cheese/day, 20% fruit/daily, 20% vegetables/salads 2-3 times/week, 60% legumes once/week, 50% rice/or pasta once/week, 60% red meat 2-3 times per week, 56% white meat once/week, 50% seafood rarely, 42% traditional Greek meals once/week, 10% nuts 2-3 times/week, 40% olive oil once/day, 30% fast food once/week, 30% pies once/week, 40% sweets 2-3 times/week, 30% salty snacks once/week, 14% soft drinks once/week.

Regarding fruit intake, at baseline only 44% of children consumed one fruit/day which decreased to 25% at follow-up, and 25% consumed fruit juice once/day at both time-points. As for cereals

including bread, 27% of children consumed cereals once/day which increased to 36% at follow-up. With respect to fish intake, at baseline 36% lean/or fatty fish once/week which increased to 34% at a frequency of 2-3 times/week particularly for fatty fish as a result of the dietary intervention.

Frequency of food intake from FFQs per intervention group at baseline is shown in Table 3 and for six months in Table 4.

Table 3 Frequency of food intake per intervention and control group at baseline

FFQ (Baseline)	How many times in the last month did your child consume.....?																
	Group																
	Intervention								Control								
Food item /Frequency of intake (%)	Never/ rarely	1-3 x/ month	1x/ week	2-3 x/ week	4-6 x/ week	1x/ day	2-3 x/ day	≥ 4 x/ day	Never/ rarely	1-3 x/ month	1x/ week	2-3 x/ week	4-6 x/ week	1x/ day	2-3 x/ day	≥ 4 x/ day	P*
Milk	3.2	0.0	3.2	9.7	9.7	38.7	32.3	3.2	0.0	0.0	3.0	3.0	12.1	48.5	33.3	0.0	0.71
Chocolate Milk	64.5	9.7	6.5	6.5	0.0	9.7	3.2	0.0	54.6	6.1	18.2	0.0	6.1	6.1	9.1	0.0	0.28
Yogurt	16.1	16.1	22.6	38.7	3.2	0.0	3.2	0.0	12.1	24.2	18.2	30.3	6.1	9.1	0.0	0.0	0.50
Cheese	9.7	3.2	9.7	22.6	22.6	19.4	12.9	0.0	9.1	6.1	15.1	18.2	21.2	27.3	3.0	0.0	0.78
Fruit	3.2	3.2	3.2	22.6	9.7	48.4	9.7	0.0	3.0	6.1	3.0	24.2	18.2	39.4	6.1	0.0	0.95
Fruit juice	6.5	0.0	12.9	35.5	9.7	35.5	0.0	0.0	15.1	9.1	6.1	36.4	6.1	21.2	6.1	0.0	0.24
Stewed vegetables in sauce	9.7	6.5	51.6	32.3	0.0	0.0	0.0	0.0	15.1	18.2	39.4	27.3	0.0	0.0	0.0	0.0	0.42
Boiled vegetables	29.0	19.4	25.8	19.3	3.2	3.2	0.0	0.0	36.4	15.2	21.2	21.2	3.0	3.0	0.0	0.0	0.98
Salads	19.4	19.4	3.2	38.7	6.5	12.9	0.0	0.0	15.1	6.1	15.1	36.4	12.1	15.1	0.0	0.0	0.36
Legumes	3.2	3.2	54.8	38.7	0.0	0.0	0.0	0.0	3.0	12.1	57.6	27.3	0.0	0.0	0.0	0.0	0.52
Cereals	3.2	0.0	12.9	16.1	32.6	29.0	3.2	3.2	12.1	0.0	6.1	33.3	21.2	24.2	3.0	0.0	0.39
Pasta	0.0	0.0	51.6	32.3	12.9	0.0	3.2	0.0	3.0	6.1	51.5	36.4	3.0	0.0	0.0	0.0	0.31
Rice	6.4	19.4	48.4	22.6	0.0	0.0	3.2	0.0	15.1	30.3	45.5	9.1	0.0	0.0	0.0	0.0	0.30
Red Meat	0.0	3.2	25.8	67.7	0.0	3.2	0.0	0.0	0.0	6.1	33.3	51.5	9.1	0.0	0.0	0.0	0.27
White Meat	0.0	0.0	61.3	35.5	0.0	3.2	0.0	0.0	0.0	6.1	51.5	39.4	3.0	0.0	0.0	0.0	0.38
Traditional Meals	0.0	29.2	51.6	16.1	0.0	0.0	0.0	3.2	12.1	54.6	30.3	3.0	0.0	0.0	0.0	0.0	0.02
Seafood	54.8	32.3	9.7	3.2	0.0	0.0	0.0	0.0	63.6	27.3	9.1	0.0	0.0	0.0	0.0	0.0	0.71
Lean Fish	6.5	38.7	48.4	6.5	0.0	0.0	0.0	0.0	36.4	33.3	24.2	6.1	0.0	0.0	0.0	0.0	0.03
Fatty Fish	6.5	41.9	45.2	6.5	0.0	0.0	0.0	0.0	39.4	30.3	27.3	3.0	0.0	0.0	0.0	0.0	0.02
Margarine	54.8	9.7	3.2	19.3	6.5	6.5	0.0	0.0	63.6	9.1	9.1	12.1	6.1	0.0	0.0	0.0	0.58
Nuts	51.6	35.5	9.7	0.0	3.2	0.0	0.0	0.0	33.3	42.4	6.1	18.2	0.0	0.0	0.0	0.0	0.08
Olive oil	0.0	0.0	0.0	6.5	32.3	45.2	9.7	6.5	3.0	0.0	3.0	3.0	18.2	45.5	18.2	9.1	0.61
Fast Food	16.1	45.2	32.3	6.5	0.0	0.0	0.0	0.0	18.2	36.4	33.3	9.1	0.0	3.0	0.0	0.0	0.84
Pies	32.3	32.3	19.4	12.9	0.0	3.2	0.0	0.0	15.1	21.2	33.3	24.2	0.0	6.1	0.0	0.0	0.26
Sweets	3.2	12.9	9.7	41.9	22.6	9.7	0.0	0.0	3.0	9.1	9.1	42.4	24.2	12.1	0.0	0.0	0.99
Salty Snacks	25.8	32.3	25.8	16.1	0.0	0.0	0.0	0.0	18.2	24.2	24.2	30.3	3.0	0.0	0.0	0.0	0.54
Soft/Energy drinks	74.2	16.1	9.7	0.0	0.0	0.0	0.0	0.0	54.6	24.2	3.0	18.2	0.0	0.0	0.0	0.0	0.04

P-values estimated using Chi Square test with 5% significance level.

Table 3 shows that at baseline, the control consumed more soft drinks (2-3 times/week) than the intervention group ($p = 0.041$; control vs intervention, 18.2% vs 0.0% at 2-3x/weekly); However, the intervention group consumed more fatty fish weekly ($p = 0.020$; intervention vs control: 45.2% vs 27.3%), lean fish ($p = 0.026$; 48.4% vs 24.2%) and traditional meals ($p = 0.017$; 51.6% vs 30.3%) than the control.

Table 4. Frequency of food intake per intervention and control group at six months

FFQ(Six months)		How many times in the last month did your child consume.....?															
		Group															
		Intervention							Control								
Food item /Frequency of intake (%)	Never/ rarely	1-3 x/ month	1x/ week	2-3 x/ week	4-6 x/ week	1x/ day	2-3 x/ day	≥ 4 x/ day	Never/ rarely	1-3 x/ month	1x/ week	2-3 x/ week	4-6 x/ week	1x/ day	2-3 x/ day	≥ 4 x/ day	P*
Milk	0.0	0.0	0.0	19.4	9.7	38.7	29.0	3.2	3.0	0.0	0.0	6.1	15.2	33.3	42.4	0.0	0.35
Chocolate Milk	64.5	16.1	6.5	0.0	0.0	9.7	3.2	0.0	60.6	15.2	6.1	0.0	9.1	0.0	9.1	0.0	0.23
Yogurt	29.0	12.9	16.1	32.3	6.5	3.2	0.0	0.0	18.2	27.3	21.2	15.2	9.1	9.1	0.0	0.0	0.34
Cheese	6.5	0.0	6.5	25.8	29.0	29.0	3.2	0.0	6.1	12.1	6.1	30.3	27.3	12.1	6.1	0.0	0.38
Fruit	3.2	0.0	3.2	16.1	29.0	38.7	9.7	0.0	0.0	15.2	0.0	27.3	27.3	12.1	18.2	0.0	0.04
Fruit juice	3.2	6.5	12.9	45.2	6.5	22.6	3.2	0.0	3.0	12.1	6.1	39.4	9.1	27.3	3.0	0.0	0.94
Stewed vegetables in sauce	12.9	19.4	38.7	29.0	0.0	0.0	0.0	0.0	21.1	9.1	45.5	24.2	0.0	0.0	0.0	0.0	0.54
Boiled vegetables	29.0	19.4	29.0	22.6	0.0	0.0	0.0	0.0	42.4	15.2	30.3	9.1	0.0	3.0	0.0	0.0	0.44
Salads	16.1	3.2	12.9	38.7	12.9	16.1	0.0	0.0	12.1	0.0	6.1	36.4	18.2	24.2	3.0	0.0	0.70
Legumes	3.2	0.0	67.7	29.0	0.0	0.0	0.0	0.0	6.1	21.2	54.5	18.2	0.0	0.0	0.0	0.0	0.04
Cereals	3.2	3.2	3.2	32.3	9.7	35.5	9.7	3.2	9.1	0.0	3.0	18.2	27.3	36.4	6.1	0.0	0.41
Pasta	0.0	3.2	51.6	38.7	6.5	0.0	0.0	0.0	0.0	15.2	39.4	45.5	0.0	0.0	0.0	0.0	0.15
Rice	12.9	16.1	45.2	19.4	6.5	0.0	0.0	0.0	9.1	27.3	54.5	9.1	0.0	0.0	0.0	0.0	0.32
Red Meat	0.0	6.5	38.7	51.6	3.2	0.0	0.0	0.0	0.0	12.1	18.2	57.6	9.1	3.0	0.0	0.0	0.30
White Meat	0.0	9.7	61.3	29.0	0.0	0.0	0.0	0.0	0.0	15.2	54.5	24.2	6.1	0.0	0.0	0.0	0.47
Traditional Meals	0.0	38.7	54.8	6.5	0.0	0.0	0.0	0.0	9.1	51.5	30.3	9.1	0.0	0.0	0.0	0.0	0.12
Seafood	54.8	35.5	9.7	0.0	0.0	0.0	0.0	0.0	39.4	51.5	9.1	0.0	0.0	0.0	0.0	0.0	0.42
Lean Fish	51.6	22.6	6.5	16.1	3.2	0.0	0.0	0.0	18.2	45.5	32.3	3.0	0.0	0.0	0.0	0.0	0.00
Fatty Fish	0.0	3.2	25.8	67.7	3.2	0.0	0.0	0.0	33.3	42.4	21.2	3.0	0.0	0.0	0.0	0.0	0.00
Margarine	51.6	6.5	19.4	16.1	0.0	6.5	0.0	0.0	72.7	9.1	3.0	6.1	6.1	3.0	0.0	0.0	0.11
Nuts	41.9	41.9	9.7	3.2	3.2	0.0	0.0	0.0	33.3	42.4	12.1	12.1	0.0	0.0	0.0	0.0	0.54
Olive oil	3.2	0.0	3.2	9.7	12.9	48.4	12.9	9.7	3.0	3.0	0.0	0.0	33.3	30.3	24.2	6.1	0.15
Fast Food	6.5	54.8	35.5	3.2	0.0	0.0	0.0	0.0	12.1	42.4	33.3	12.1	0.0	0.0	0.0	0.0	0.44
Pies	6.5	51.6	29.0	9.7	3.2	0.0	0.0	0.0	12.1	24.2	39.4	21.2	0.0	3.0	0.0	0.0	0.18
Sweets	0.0	9.7	29.0	45.2	9.7	6.5	0.0	0.0	9.1	3.0	15.2	42.4	9.1	18.2	3.0	0.0	0.23
Salty Snacks	9.7	54.8	25.8	6.5	0.0	3.2	0.0	0.0	12.1	33.3	36.4	9.1	6.1	3.0	0.0	0.0	0.50
Soft/Energy drinks	61.3	9.7	12.9	12.9	0.0	3.2	0.0	0.0	42.4	24.2	15.2	18.2	0.0	0.0	0.0	0.0	0.35

*P values estimated using Chi Square Test significant at the 5% level.

From Table 4 it is apparent that at six months the intervention group consumed more fruit daily (intervention vs control: 38.7% vs 12.1% once daily; $p = 0.042$), legumes weekly (67.7% vs 54.5% ; $p = 0.044$) and fatty fish 2-3 times/week (67.7% vs 3.0%; $p < 0.001$) compared to the control due to the nature of the dietary intervention. Although the control consumed more lean fish weekly as expected (intervention v control: 6.5% vs 32.3%; $p = 0.002$).

Frequency of food group intake in times per day was calculated from responses in FFQs and is displayed in Table 5 for baseline.

Table 5. Frequency of food group intake (times per day) per intervention and control group at baseline

Frequency of food group intake (Baseline)					
Food Group (times/day)	Intervention		Control		<i>P</i> ^b
	Mean	SD	Mean	SD	
Dairy	2.71	1.34	2.65	1.45	0.64
Fruit	1.51	0.78	1.35	0.84	0.29
Vegetable	0.70	0.54	0.73	0.56	0.85
Starch	1.41	1.18	0.93	0.48	0.22
Legumes	0.22	0.12	0.19	0.11	0.08
Meat	0.56	0.31	0.54	0.26	0.77
Seafood	0.05	0.07	0.03	0.05	0.45
Fish	0.23	0.13	0.15	0.15	0.00
Nuts	0.06	0.13	0.10	0.13	0.09
Fats	1.32	.93	1.62	1.20	0.21
Fast food	0.10	0.08	0.13	0.18	0.64
Sweets	0.43	0.29	0.46	0.30	0.63
Savoury snacks	0.24	0.24	0.39	0.34	0.03

In bold statistically significant *p*-values

^b Mann-Whitney

Total sample baseline *N* = 64; Intervention group *n* = 31; Control group *n* = 33

Table 5 shows that at baseline there was a significant difference in frequency of fish intake times per day for the intervention group as compared to the control (intervention vs control (mean) 0.23 vs 0.15 times/day; *p* = 0.002). However a higher frequency of savoury snack intake was found for the control as compared to the intervention group (control vs intervention: 0.39 vs 0.24 times/day; *p* = 0.032)

Frequency of food group intake (in times per day) by intervention and control group at six months is shown in Table 6.

Table 6. Frequency of food group intake (times per day) per intervention and control group at six months

Frequency of food group intake (Six months)					
Food Group (times/day)	Intervention		Control		<i>P</i> ^b
	Mean	SD	Mean	SD	
Dairy	2.48	1.20	2.67	1.44	0.90
Fruit	1.46	0.98	1.38	1.03	0.48
Vegetables	0.72	0.43	0.86	0.65	0.49
Legumes	0.20	0.11	0.16	0.11	0.03
Starch	1.37	0.99	1.13	0.53	0.59
Meat	0.46	0.21	0.55	0.31	0.27
Seafood	0.04	0.05	0.05	0.04	0.29
Fish	0.41	0.25	0.16	0.13	0.00
Nuts	0.08	0.14	0.09	0.11	0.38
Fat	1.55	1.23	1.58	1.03	0.66
Fast food	0.10	0.06	0.12	0.10	0.65
Sweets	0.34	0.25	0.50	0.48	0.22
Savoury	0.26	0.23	0.36	0.33	0.08
Snacks					

^bMann-Whitney test

Significant p-values in bold

Total sample *N* = 64; Intervention group *n* = 31, Control *n* = 33

Table 6 shows that at six months there was a significant increase in the frequency of intake of legumes (intervention vs control (mean): 0.20 vs 0.16 times/day; *p* = 0.034) and fish (intervention vs control (mean): 0.41 vs 0.16 times/day; *p* = 0.000) in the intervention group as compared to the control.

Comparison of frequency of food group intake (times per day) from FFQs according to group from baseline to six months is shown in Table 7.

Table 7. Comparison of frequency of food group intake (times per day) from FFQs by intervention and control group from baseline to six months

COMPARISON OF FREQUENCY OF FOOD GROUP INTAKE (IN TIMES PER DAY) FROM FFQS BY GROUP (BASELINE VS SIX MONTHS)						
	Intervention			Control		
Frequency of food group intake (times/day)	Baseline Mean± SD	Six months Mean± SD	<i>P</i> *	Baseline Mean± SD	Six months Mean± SD	<i>P</i> *
Dairy	2.71±1.34	2.48±1.20	0.48	2.65±1.45	2.67±1.44	0.63
Fruit	1.51±0.78	1.46±0.98	0.63	1.35±0.84	1.38±1.03	0.78
Vegetables	0.70±0.54	0.72±0.43	0.39	0.73±0.56	0.86±0.65	0.20
Legumes	0.22±0.12	1.37±0.99	0.59	0.19±0.11	1.13±0.53	0.19
Starch	1.41±1.18	0.20±0.11	0.56	0.93±0.48	0.16±0.11	0.08
Meat	0.56±0.31	0.46±0.21	0.16	0.54±0.26	0.55±0.31	0.86
Seafood	0.05±0.07	0.04±0.05	0.77	0.03±0.05	0.05±0.04	0.21
Fish	0.23±0.13	0.41±0.25	0.00	0.15±0.15	0.16±0.13	0.40
Nuts	0.06±0.13	0.08±0.14	0.53	0.10±0.13	0.09±0.11	0.39
Fats	1.32±0.93	1.55±1.23	0.14	1.62±1.20	1.58±1.03	0.68
Fast Food	0.10±0.08	0.10±0.06	0.87	0.13±0.18	0.12±0.10	0.72
Sweets	0.43±0.29	0.34±0.25	0.13	0.46±0.30	0.50±0.48	0.98
Savoury snacks	0.24±0.24	0.26±0.23	0.46	0.39±0.34	0.36±0.33	0.58

In bold are the significant p-value at 5% level.

Total sample *N* = 64; Intervention group *n* = 31; Control group *n* = 33

* *P*-value estimated using Wilcoxon rank sign test

Assessment of within group differences in frequency of food group intake (in times per day) between the two time-points showed that there was a significant difference in frequency of fish intake in the intervention group only [baseline vs six months: 0.23 vs 0.41 times/day; *p* < 0.001 (Wilcoxon rank sign test)]. No differences were observed in the control group.

Nutrient analysis of dietary intake measured from FFQs

Nutrient analysis of dietary intake measured using FFQs at both time-points was calculated using *McCance and Widdowson's "The Composition of Foods" (UK)* and Trichopoulou's "Composition tables of food and Greek dishes". Macronutrient and micronutrient intake of children's dietary intake per intervention group at both time-points is displayed in Table 8

Table 8. Total macronutrient and micronutrient intake per intervention and control group at baseline and six months.

Nutrient	Baseline		Six months	
	Group			
Macronutrients	Intervention	Control	Intervention	Control
H ₂ O (g)	1700.1	1605.7	2880.9	3114.1
Carbohydrate (g)	331.5	279.2	373.7	379.5
Protein (g)	125.1	113.4	346.8	371.4
Fat (g)	147.5	162.4	380.9	426.9
Energy(Kcal)	3006.6	2902.0	6134.6	6671.9
Fibre (g)	29.4	26.1	27.7	28.6
Fatty acid composition				
Saturated fats (g)	157.0	218.1	196.2	224.2
Monounsaturated fats (g)	274.0	290.8	274.3	308.6
Polyunsaturated fats (g)	20.6	21.1	37.9	23.3
EPA (g)	0.2	0.1	0.4	0.1
DHA (g)	0.4	0.3	0.7	0.3
Trans (g)	9.4	10.3	9.1	10.2
Cholesterol (mg)	393.9	400.4	1108.6	1209.4
Micronutrients				
Sodium (mg)	1844.3	1801.0	2869.8	3028.6
Potassium (mg)	7856.9	8136.6	6226.9	6336.0
Calcium (mg)	5990.6	6254.3	5638.9	6335.9
Magnesium (mg)	886.3	864.7	837.0	888.4
Phosphorus (mg)	6924.1	7016.6	6550.1	7115.8
Iron (mg)	78.9	53.8	68.0	59.2
Copper (mg)	2.8	2.4	2.5	2.5
Zinc (mg)	33.9	38.6	36.1	39.8
Chlorine (mg)	2350.6	2436.6	4480.6	4608.5
Manganese (mg)	5.3	4.6	4.9	4.9
Selenium (µg)	167.3	148.7	172.6	152.5
Iodine (µg)	707.6	678.1	729.5	697.7
Retinol (µg)	2603.5	2731.2	2464.1	2792.8
β-carotene (µg)	10029.0	10620.4	10208.6	12110.2
Vitamin D (µg)	9.8	9.1	11.6	9.3
Vitamin E (mg)	20.0	15.9	21.0	22.7
Vitamin C (mg)	495.1	463.3	480.8	512.6
Thiamine (mg)	2.8	2.6	2.7	2.7
Riboflavin (mg)	7.8	7.8	2.7	2.6
Niacin (mg)	32.5	25.7	32.3	28.5
Tryptophan (mg)	78.1	79.2	74.7	80.5
Vitamin B ₆ (mg)	6.9	5.3	5.2	5.3

Vitamin B ₁₂ (µg)	31.1	30.1	31.4	30.8
Folate (µg)	1076.6	1014.2	1017.4	1076.4
Pantothenate (mg)	17.0	16.9	16.1	17.2
Biotin (µg)	82.7	81.1	79.0	82.9

When investigating for differences between the groups at both time-points Mann-Whitney test showed no differences. The same outcome was obtained for within group differences after applying Wilcoxon signed rank test.

Nutrient composition of energy, macro and micronutrients and fatty acid composition for all food items and food groups at baseline per group are shown in Table 9 to Table 16.

Table 9 Nutrient analysis of macronutrients of dietary intake for intervention and control groups at baseline

NUTRIENT ANALYSIS OF MACRONUTRIENTS						
Baseline						
Food item (grams/day)	H ₂ O (g)	CHO (g)	P (g)	Fat (g)	Energy (kcal)	Fibre (g)
Milk						
Intervention: 343.45	300.86	16.49	10.99	11.68	212.94	0.00
Control: 340.65	298.41	16.35	10.90	11.50	211.20	0.00
Chocolate milk						
Intervention: 51.88	42.96	4.98	1.87	0.78	32.68	0.00
Control: 86.50	71.62	8.30	3.11	1.29	54.50	0.00
Yogurt						
Intervention: 68.48	56.09	5.34	3.90	2.05	54.10	0.00
Control: 68.36	55.99	5.33	3.89	2.05	54.00	0.00
Cheese						
Intervention: 23.20	23.26	0.67	9.81	1.07	137.10	0.00
Control: 17.70	17.75	0.51	7.49	0.81	104.60	0.00
Fruit						
Intervention: 132.37	130.27	22.50	1.23	0.26	86.00	1.95
Control: 119.43	99.12	20.30	1.11	0.24	77.52	1.75
Fruit juice						
Intervention: 149.96	133.91	13.95	0.45	0.15	54.74	0.15
Control: 132.52	118.34	12.32	0.40	0.13	48.37	0.13
Vegetables stewed						
Intervention: 46.25	35.98	2.89	0.87	4.24	51.80	1.33
Control: 39.72	30.90	2.49	0.74	3.64	44.49	1.14
Salads						
Intervention: 79.60	68.84	2.99	0.23	0.21	15.12	1.11
Control: 94.52	87.90	3.55	0.87	0.25	17.96	1.32
Vegetables boiled						
Intervention: 41.88	43.70	1.29	1.05	0.25	11.39	0.76
Control: 40.44	36.31	1.25	1.01	0.24	10.99	0.74
Legumes						
Intervention: 65.49	34.43	8.21	3.33	5.47	83.69	2.04
Control: 56.07	29.48	7.03	2.85	4.68	71.16	1.75
Cereals						
Intervention: 24.15	4.3	17.70	2.25	0.48	73.85	1.564
Control: 17.92	3.19	11.71	1.67	0.36	54.79	1.16
Pasta						
Intervention: 50.49	31.05	16.06	3.33	0.76	80.28	0.96
Control: 32.00	19.68	10.18	2.10	0.48	50.88	0.61

NUTRIENT ANALYSIS OF MACRONUTRIENTS

Food item (grams/day)	Baseline					
	H ₂ O (g)	CHO (g)	P (g)	Fat (g)	Energy (kcal)	Fibre (g)
Rice						
Intervention: 38.82	26.39	11.99	1.01	0.50	53.57	0.04
Control: 17.10	11.63	5.28	0.44	0.22	23.59	0.02
Red meat						
Intervention: 47.16	16.60	0.00	9.43	21.30	229.15	0.00
Control: 45.11	15.89	0.00	9.02	20.38	219.54	0.00
White meat						
Intervention:	25.90	0.00	8.79	1.55	49.03	0.00
Control:	25.24	0.00	8.56	1.51	47.79	0.00
Seafood						
Intervention: 7.02	4.48	0.62	1.47	0.65	13.49	0.03
Control: 4.65	2.97	0.41	0.97	0.43	8.94	0.02
Lean fish						
Intervention: 17.54	13.40	1.40	3.74	0.35	18.19	0.00
Control: 11.71	8.99	0.94	2.51	0.23	12.15	0.00
Fatty fish						
Intervention: 17.18	10.49	0.00	3.89	1.90	31.51	0.00
Control: 10.41	6.35	0.00	2.36	1.15	19.09	0.00
Traditional meals						
Intervention: 22.94	15.63	2.54	1.72	2.4	380.11	0.27
Control: 13.73	9.35	1.52	1.03	1.44	227.50	0.16
Margarine						
Intervention: 0.83	0.00	0.00	0.00	0.68	5.97	0.00
Control: 1.41	0.00	0.00	0.00	1.17	10.15	0.00
Nuts						
Intervention: 3.01	0.08	0.29	0.61	1.69	19.10	0.14
Control: 5.12	0.14	0.50	1.05	2.87	32.49	0.24
Olive oil						
Intervention: 16.43	Tr	0.00	0.00	16.27	147.70	0.00
Control: 19.34	0.00	0.00	0.00	19.15	173.87	0.00
Fast foods						
Intervention: 19.73	9.43	6.10	2.39	1.64	47.42	0.35
Control: 26.81	12.82	8.28	3.25	2.23	64.43	0.47
Pies						
Intervention: 19.11	10.57	3.14	1.53	3.40	48.25	0.31
Control: 31.31	17.31	5.15	2.50	5.57	79.06	0.50
Sweets						
Intervention: 25.82	6.48	12.37	1.51	4.69	94.29	0.34
Control: 27.89	6.99	13.36	1.63	5.07	101.85	0.37
Salty snacks						
Intervention: 8.11	0.23	4.32	0.46	2.77	42.98	0.42
Control: 12.66	0.35	6.75	0.72	4.33	67.10	0.67
Soft drinks						
Intervention: 8.52	7.56	0.92	Tr	0.00	3.45	0.00
Control: 30.08	26.71	3.25	Tr	0.00	12.18	0.00

Key: Tr- trace

Table 10. Fatty acid composition of food items per intervention and control group at baseline

NUTRIENT ANALYSIS (Fatty Acids)							
Baseline							
Food Item (grams/day)	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	Trans (g)	Cholesterol (mg)
Milk							
Intervention: 343.45	7.5	3.43	0.34			0.34	48.07
Control: 340.65	7.49	3.41	0.34			0.34	47.69
Chocolate milk							
Intervention:51.88	0.52	0.16	0.05			Tr	3.63
Control: 86.50	0.87	0.26	0.09			Tr	6.06
Yogurt							
Intervention: 68.48	1.16	0.62	0.14			N	7.53
Control: 68.36	1.16	0.61	0.14			N	7.52
Cheese							
Intervention:23.20	3.3	1.08	0.12			0.16	16.35
Control: 17.70	2.5	0.82	0.09			0.12	12.48
Fruit							
Intervention: 132.37	-	-	0.13			-	0.00
Control: 119.43	-	-	0.12			-	0.00
Fruit juice							
Intervention: 149.96	Tr	Tr	Tr			-	0.00
Control: 132.52	Tr	Tr	Tr			-	0.00
Vegetables stewed							
Intervention: 46.25	0.62	2.81	0.56			-	0.00
Control: 39.72	0.52	2.41	0.47			-	0.00
Salads							
Intervention: 79.60	0.08	0.08	0.18			-	0.00
Control: 94.52	0.09	0.09	0.22			-	0.00
Vegetables boiled							
Intervention: 41.88	3.41	0.04	0.14			0.00	0.00
Control: 40.44	3.29	0.04	0.14			0.00	0.00
Legumes							
Intervention: 65.49	0.77	2.59	0.64			0.00	2.84
Control: 56.07	0.66	2.23	0.55			0.00	2.42
Cereals							
Intervention: 24.15	0.08	0.08	0.19			Tr	0.00
Control: 17.92	0.06	0.06	0.15			Tr	0.00
Pasta							
Intervention: 50.49	0.15	0.15	0.20			-	N
Control: 32.00	0.10	0.10	0.13			-	N
Rice							
Intervention: 38.82	0.12	0.12	0.19			0.00	0.00
Control: 17.10	0.05	0.05	0.09			0.00	0.00
Red meat							
Intervention: 47.16	10.29	8.68	0.89			1.57	49.05
Control: 45.11	9.85	8.30	0.86			1.50	46.91
White meat							
Intervention:36.87	0.55	0.54	0.36			0.04	33.67
Control: 35.93	0.53	0.53	0.35			0.04	32.81
Seafood							
Intervention: 7.02	0.16	0.42	0.10			0.00	11.58
Control:4.65	0.11	0.28	0.07			0.00	7.67
Lean fish							
Intervention: 17.54	0.07	0.11	0.11	0.02	0.04	0.00	9.96
Control: 11.71	0.05	0.07	0.08	0.01	0.03	0.00	6.65
Fatty fish							
Intervention: 17.18	0.49	1.21	0.59	0.09	0.16	0.00	13.01
Control: 10.41	0.29	0.73	0.36	0.06	0.09	0.00	7.88

NUTRIENT ANALYSIS (Fatty Acids)							
				Baseline			
Food Item (grams/day)	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	Trans (g)	Cholesterol (mg)
Traditional meals							
Intervention: 22.94	0.75	1.20	0.27			0.09	5.73
Control: 13.73	0.45	0.72	0.16			0.05	3.43
Margarine							
Intervention: 0.83	0.29	0.30	0.04			0.10	2.37
Control: 1.41	0.49	0.51	0.08			0.17	4.02
Nuts							
Intervention: 3.01	0.22	0.77	0.62			0.00	0.00
Control: 5.12	0.37	1.32	1.05			0.00	0.00
Olive oil							
Intervention: 16.43	2.35	11.99	1.35			0.00	0.00
Control: 19.34	2.77	14.12	1.58			0.00	0.00
Fast foods							
Intervention: 19.73	0.61	0.69	0.22			0.04	4.80
Control: 26.81	0.82	0.93	0.30			0.05	6.52
Pies							
Intervention: 19.11	1.02	1.79	0.24			-	12.23
Control: 31.31	1.67	2.94	0.39			-	20.04
Sweets							
Intervention: 25.82	2.29	1.28	0.28			0.16	5.16
Control: 27.89	2.47	1.38	0.29			0.17	5.58
Salty snacks							
Intervention: 8.11	1.14	1.11	0.40			N	0.00
Control: 12.06	1.77	1.73	0.63			N	0.00
Soft drinks							
Intervention: 8.52	0.00	0.00	0.00			0.00	0.00
Control: 30.08	0.00	0.00	0.00			0.00	0.00

Key: Sat-saturated fats; MUFA- monounsaturated fatty acids; PUFA-polyunsaturated fatty acids, Trans-trans fatty acids; EPA-Eicosapentaenoic acid; DHA- Docosahexaenoic acid; Tr-trace; N-Nil

Table 11. Mineral composition of baseline data by intervention and control group

NUTRIENT ANALYSIS (MINERALS)														
FOOD (grams/day)	Baseline													
	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β- Carotene (µg)
Milk														
Intervention: 343.45	147.86	53.20	40.52	37.77	319.79	0.10	Tr	1.37	305.58	0.34	3.43	106.44	113.3	68.67
Control: 340.65	146.48	52.80	401.97	37.47	316.80	0.10	Tr	1.36	303.17	0.34	3.41	105.60	112.41	68.13
Chocolate milk														
Intervention: 51.88	23.34	106.87	59.66	9.86	55.51	0.32	0.03	0.26	57.02	Tr	N	N	4.15	4.15
Control: 86.50	38.90	178.19	99.47	16.44	92.56	0.54	0.05	0.43	95.15	Tr	N	N	17.3	6.92
Yogurt														
Intervention: 68.48	54.78	191.74	136.96	13.01	116.42	0.07	Tr	0.48	116.42	Tr	1.37	43.14	19.17	14.38
Control: 68.36	54.69	191.4	136.72	12.99	116.21	0.07	Tr	0.48	116.21	Tr	1.37	43.10	19.14	14.36
Cheese														
Intervention: 23.20	565.15	42.60	151.96	12.53	182.81	0.12	0.02	0.90	909.44	Tr	2.78	2.78	94.66	49.88
Control: 17.70	431.17	32.57	115.94	9.56	139.48	0.09	0.01	0.69	693.84	Tr	2.12	2.12	72.22	38.06
Fruit														
Intervention: 132.37	3.53	281.60	25.15	20.74	24.71	0.21	0.07	0.20	3.71	0.53	1.32	4.37	0.00	28.68
Control: 119.43	3.19	254.02	22.69	18.71	22.29	0.19	0.07	0.18	33.44	0.48	1.19	3.94	0.00	25.88
Fruit juice														
Intervention: 149.96	4.00	318.90	28.49	23.54	28.04	0.24	0.09	0.22	41.99	0.61	1.49	4.95	0.00	32.50
Control: 132.52	3.54	281.83	25.18	20.80	24.78	0.21	0.08	0.19	37.10	0.54	1.32	4.37	0.00	28.72
Vegetables stewed														
Intervention: 46.25	3.39	149.10	24.51	12.79	19.27	0.41	0.05	0.15	2.31	0.09	0.46	Tr	0.00	226.00
Control: 39.72	2.91	128.03	21.05	10.99	16.55	0.36	0.04	0.13	1.99	0.08	0.39	Tr	0.00	194.10

NUTRIENT ANALYSIS (MINERALS)														
Baseline														
FOOD (grams/day)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β- Carotene (µg)
Salads														
Intervention: 79.60	7.00	157.60	19.26	4.94	24.3	0.35	0.01	0.11	28.01	0.13	0.79	1.75	0.00	2275.92
Control: 94.52	8.31	187.14	22.87	5.86	27.78	0.42	0.01	0.13	33.27	0.15	0.95	2.08	0.00	2702.51
Vegetables boiled														
Intervention: 41.88	20.73	103.86	22.19	7.71	21.78	0.37	0.00	0.17	8.90	0.16	0.42	0.84	0.00	637.08
Control: 40.44	20.06	100.30	21.43	7.48	21.02	0.36	0.00	0.16	8.61	0.15	0.40	0.81	0.00	615.17
Legumes														
Intervention: 65.49	8.02	0.05	23.45	27.7	61.76	1.37	0.20	0.69	8.10	0.33	7.53	3.93	0.00	48.79
Control: 56.07	6.87	184.08	20.07	23.72	52.87	1.18	0.17	0.59	6.94	0.28	6.45	3.36	0.00	41.77
Cereals														
Intervention: 24.15	142.5	76.30	18.00	17.82	53.47	13.18	0.05	0.39	266.2	0.19	1.33	1.69	0.00	0.00
Control: 17.92	106.37	56.63	13.37	13.22	39.67	9.78	0.03	0.29	197.84	0.14	0.98	1.25	0.00	0.00
Pasta														
Intervention: 50.49	8.08	24.74	18.68	9.59	43.42	0.40	0.23	0.40	14.64	0.20	6.56	18.18	0.00	0.00
Control: 32.00	5.12	15.68	11.84	6.08	27.52	0.26	0.15	0.26	9.28	0.13	4.16	11.52	0.00	0.00
Rice														
Intervention: 38.82	0.39	20.96	6.99	4.27	20.96	0.08	0.05	0.27	1.55	0.08	1.94	1.94	0.00	0.00
Control: 17.10	0.17	9.20	3.10	1.88	9.23	0.03	0.02	0.12	0.68	0.03	0.86	0.86	0.00	0.00
Red meat														
Intervention: 47.16	30.18	130.48	5.81	8.96	78.60	0.66	0.27	1.19	29.24	0.00	1.73	4.09	3.30	Tr
Control: 45.11	28.87	124.8	5.56	8.57	75.18	0.63	0.26	1.14	27.98	0.00	1.66	3.91	3.15	Tr
White meat														
Intervention: 36.87	30.45	113.06	7.74	8.23	63.89	0.56	0.02	0.84	29.44	0.01	5.78	2.58	7.33	Tr
Control: 35.93	24.68	110.19	7.55	8.02	62.27	0.55	0.02	0.82	28.85	0.01	5.63	2.51	7.19	Tr

Intervention: 19.11	69.18	43.19	33.92	4.78	25.99	0.25	-	0.14	-	-	-	-	15.29	173.33
Control: 31.31	113.34	70.76	55.57	7.80	42.58	0.41	-	0.23	-	-	-	-	25.00	283.98
Sweets														
Intervention: 25.82	48.71	45.99	23.80	5.51	35.29	0.27	0.03	0.15	94.24	0.08	1.16	6.20	22.97	7.44
Control: 27.89	52.62	49.64	25.70	5.95	38.12	0.29	0.03	0.16	101.79	0.08	1.26	6.69	24.82	8.03
Salty snacks														
Intervention: 8.11	64.88	85.97	2.35	4.62	8.92	0.11	0.01	0.05	106.24	0.03	0.08	N	0.00	0.16
Control: 12.66	101.28	134.2	3.67	7.21	13.92	0.18	0.02	0.08	165.84	0.05	0.13	N	0.00	0.25
Soft drinks														
Intervention: 8.52	0.97	1.07	0.45	0.21	0.93	Tr	Tr	Tr	0.54	Tr	Tr	Tr	0.00	39.57
Control: 30.08	3.45	3.76	1.58	0.75	3.30	Tr	Tr	Tr	1.89	Tr	Tr	Tr	0.00	139.72

Table 12. Vitamin composition of dietary data for intervention and control group at baseline

NUTRIENT ANALYSIS (VITAMINS)												
FOOD ITEM (grams/day)	Baseline											
	Vitamin D (µg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tryptophan (mg)	B6 (mg)	B12 (µg)	Folate (µg)	Pantothenate (mg)	Biotin (µg)	Vitamin C (mg)
Milk												
Intervention: 343.45	Tr	0.27	0.10	0.79	0.69	2.06	0.21	3.09	27.47	1.99	8.58	6.87
Control: 340.65	Tr	0.92	0.10	0.78	0.68	2.04	0.20	3.06	27.25	1.98	8.52	6.81
Chocolate milk												
Intervention: 51.88	0.00	0.01	0.02	0.09	0.05	0.41	0.02	0.05	0.04	0.02	1.14	Tr
Control: 86.50	0.00	0.03	0.023	0.15	0.08	0.69	0.03	0.09	1.73	0.26	1.90	Tr
Yogurt												
Intervention: 68.48	0.00	0.03	0.04	0.18	0.14	0.89	0.07	0.14	12.33	0.34	1.78	0.68
Control: 68.36	0.00	0.03	0.04	0.18	0.14	0.89	0.07	0.14	12.30	0.34	1.78	0.68
Cheese												
Intervention: 23.20	0.18	0.27	0.02	0.13	0.07	2.23	0.04	0.74	14.38	0.17	0.97	Tr
Control: 17.70	0.14	0.21	0.01	0.10	0.05	1.69	0.03	0.57	10.97	0.13	0.74	Tr
Fruit												
Intervention: 132.37	0.00	0.34	0.08	0.05	0.53	0.17	0.20	0.00	22.07	0.32	2.12	34.81
Control: 119.43	0.00	0.31	0.07	0.05	0.48	0.16	0.18	0.00	19.91	0.29	1.91	31.41
Fruit juice												
Intervention: 149.96	0.00	0.13	0.06	0.01	0.22	0.15	0.06	0.00	14.99	0.10	1.49	47.99
Control: 132.52	0.00	0.11	0.05	0.01	0.19	0.13	0.05	0.00	13.2	0.09	1.33	42.40
Vegetables stewed												
Intervention: 46.25	0.00	0.83	0.07	0.02	0.42	0.21	0.10	0.00	20.35	0.11	0.23	10.48
Control: 39.72	0.00	0.72	0.06	0.02	0.36	0.18	0.08	0.00	17.48	0.09	0.20	9.00
Salads												
Intervention: 79.60	0.00	0.41	0.06	0.02	0.51	0.18	0.09	0.00	27.22	0.19	0.60	12.26
Control: 94.52	0.00	0.49	0.07	0.02	0.60	0.21	0.10	0.00	32.33	0.23	0.72	14.56

NUTRIENT ANALYSIS (VITAMINS)

FOOD ITEM (grams/day)	Baseline											
	Vitamin D (µg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tryptophan (mg)	B6 (mg)	B12 (µg)	Folate (µg)	Pantothenate (mg)	Biotin (µg)	Vitamin C (mg)
Vegetables boiled												
Intervention:41.88	0.00	0.31	0.02	0.01	0.20	0.22	0.08	0.05	28.23	0.09	0.23	39.78
Control: 40.44	0.00	29.52	0.02	0.01	0.19	0.21	0.08	0.04	27.26	0.08	0.22	38.42
Legumes												
Intervention:65.49	0.00	0.50	0.11	0.04	1.15	1.10	0.11	0.00	45.38	1.34	1.37	2.95
Control: 56.07	0.00	0.43	0.09	0.04	0.98	0.94	0.09	0.00	38.86	1.14	1.18	2.52
Cereals												
Intervention: 24.15	0.20	0.57	0.13	0.13	2.03	0.46	0.21	0.31	45.46	0.13	1.20	15.90
Control: 17.92	0.15	0.42	0.10	0.09	1.5	0.34	0.16	0.23	33.73	0.10	0.89	11.80
Pasta												
Intervention: 50.49	0.00	Tr	0.03	0.01	0.35	0.50	0.01	0.00	2.02	Tr	Tr	0.00
Control: 32.00	0.00	Tr	0.02	0.01	0.22	0.32	0.01	0.00	1.28	Tr	Tr	0.00
Rice												
Intervention:38.82	0.00	Tr	0.00	Tr	0.35	0.23	2.72	0.00	2.72	0.04	0.39	0.78
Control: 17.10	0.00	Tr	0.00	Tr	0.15	0.1	0.01	0.00	1.20	0.02	0.17	0.34
Red meat												
Intervention: 47.16	0.26	0.07	0.04	0.1	1.76	1.49	0.11	0.94	5.5	0.42	0.79	0.00
Control: 45.11	0.25	0.07	0.04	0.1	1.68	1.43	0.11	0.90	5.26	0.40	0.75	0.00
White meat												
Intervention:36.87	0.07	0.03	0.03	0.08	2.23	1.83	0.12	0.73	4.17	0.36	0.86	0.00
Control: 35.93	0.07	0.03	0.02	0.07	2.27	1.78	0.12	0.72	4.07	0.35	0.84	0.00
Seafood												
Intervention: 7.02	0.01	0.03	0.01	0.21	0.00	0.00	1.89	0.7	1.05	0.02	0.07	0.35
Control:4.65	0.00	0.02	0.01	0.13	0.04	0.17	0.012	0.47	0.69	0.02	0.05	0.23

NUTRIENT ANALYSIS (VITAMINS)

Baseline

FOOD ITEM (grams/day)	Vitamin D (µg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tryptophan (mg)	B6 (mg)	B12 (µg)	Folate (µg)	Pantothenate (mg)	Biotin (µg)	Vitamin C (mg)
Lean fish												
Intervention: 17.54	Tr	0.10	0.02	0.02	0.87	0.72	0.07	0.43	2.19	0.06	0.52	Tr
Control: 11.71	Tr	0.07	0.01	0.01	0.58	0.48	0.05	0.29	1.46	0.04	0.35	Tr
Fatty fish												
Intervention: 17.18	1.29	0.21	0.03	0.04	1.07	0.71	0.08	1.37	2.11	0.19	1.07	Tr
Control: 10.41	0.78	0.13	0.02	0.02	0.65	0.43	0.05	0.83	1.28	0.12	0.65	Tr
Traditional meals												
Intervention: 22.94	0.07	0.23	0.02	0.03	0.34	0.34	0.04	0.23	1.83	0.11	0.46	4.24
Control: 13.73	0.04	0.14	0.01	0.02	0.20	0.21	0.02	0.14	1.09	0.07	0.27	2.54
Margarine												
Intervention: 0.83	0.07	0.04	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 1.41	0.11	0.06	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Nuts												
Intervention: 3.01	0.00	0.23	0.01	0.01	0.14	0.13	0.01	0.00	1.7	0.04	1.70	0.00
Control: 5.12	0.00	0.39	0.01	0.01	0.24	0.22	0.02	0.00	2.89	0.06	2.89	0.00
Olive oil												
Intervention: 16.43	0.00	0.84	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 19.34	0.00	0.99	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Fast foods												
Intervention: 19.73	0.06	0.16	0.04	0.02	0.37	0.47	0.02	0.17	3.10	0.09	0.49	0.39
Control: 26.81	0.08	0.22	0.05	0.03	0.51	0.64	0.27	0.23	4.16	0.12	0.67	0.54
Pies												
Intervention: 19.11	-	0.24	0.02	0.03	N	N	0.02	N	N	N	N	1.53
Control: 31.31	-	0.39	0.03	0.05	N	N	0.04	N	N	N	N	2.50

Sweets

Intervention: 25.82	0.09	0.31	0.02	0.05	0.16	0.01	0.01	0.15	5.53	0.16	1.11	0.26
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Control: 27.89	0.09	0.33	0.03	0.06	0.18	0.37	0.01	0.17	5.97	0.18	1.19	0.28
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Salty snacks

Intervention: 8.11	0.00	0.48	0.02	0.01	0.26	0.10	0.07	0.00	2.40	0.07	N	2.83
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Control: 12.66	0.00	0.76	0.03	0.01	0.40	0.16	0.10	0.00	3.80	0.12	N	4.43
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Soft drinks

Intervention: 8.52	0.00	0.00	Tr	Tr	Tr	Tr	Tr	0.00	0.08	Tr	Tr	0.38
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Control: 30.08	0.00	0.00	Tr	Tr	Tr	Tr	Tr	0.00	0.30	Tr	Tr	1.35
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Key: Tr- Trace; N- Nil

NUTRIENT ANALYSIS OF FOOD GROUPS AT BASELINE

Table 13 Macronutrient composition of main food groups per intervention and control group at baseline

NUTRIENT ANALYSIS OF FOOD GROUPS						
Baseline						
FOOD GROUP (grams/day)	H ₂ O (g)	CHO (g)	P (g)	Fat (g)	Energy (kcal)	Fibre (g)
Dairy products						
Intervention: 486.91	398.78	37.98	27.71	14.60	384.66	0.00
Control: 513.21	420.32	40.03	29.25	15.39	405.44	0.00
Fruit						
Intervention: 282.33	486.45	74.25	3.47	0.85	286.5	4.43
Control: 251.94	434.10	66.26	3.09	0.76	255.64	3.96
Vegetables						
Intervention: 167.74	440.48	18.17	8.37	17.16	251.61	10.68
Control: 174.68	458.70	18.92	8.72	17.87	262.00	11.13
Legumes						
Intervention: 65.49	34.43	8.21	3.33	5.47	83.69	2.04
Control: 56.07	29.48	7.03	2.85	4.68	71.16	1.75
Starch						
Intervention: 113.47	167.23	145.31	21.01	5.45	684.11	9.62
Control: 67.02	98.75	85.81	12.41	3.21	403.99	5.68
Meat						
Intervention: 84.03	88.65	6.72	36.83	41.48	520.71	0.00
Control: 81.04	85.49	6.48	35.52	40.00	502.18	0.00
Seafood						
Intervention: 7.02	4.48	0.62	1.47	0.65	13.49	0.03
Control: 4.65	2.97	0.41	0.97	0.43	8.94	0.02
Fish						
Intervention: 34.72	47.86	2.78	15.31	4.53	99.70	0.00
Control: 22.12	30.49	1.99	9.70	2.89	63.52	0.00
Fats						
Intervention: 20.28	0.03	0.00	0.00	36.87	328.33	0.00
Control: 25.87	0.04	0.00	0.00	47.03	418.83	0.00
Fast foods						
Intervention: 19.73	9.43	6.10	2.39	1.64	47.42	0.35
Control: 26.81	12.82	8.28	3.25	2.23	64.43	0.47
Sweets						
Intervention: 25.82	6.48	12.37	1.51	4.69	94.29	0.34
Control: 27.89	6.99	13.36	1.63	5.07	101.85	0.37
Savoury snacks						
Intervention: 27.22	15.81	18.99	3.73	14.15	212.99	1.88
Control: 43.97	25.55	30.67	6.02	22.86	344.06	3.03
Total macronutrient intake:						
Intervention (grams/d)	1700.11	294.04	125.13	147.54	3006.60	29.37
Control	1605.70	279.24	113.41	162.42	2902.04	26.10

Food groups were calculated as follows:

Dairy products= Σ [milk+ chocolate milk+ cheese+ yogurt]

Fruits= Σ [fruit + fruit juice]

Vegetables= Σ [stewed vegetables + boiled vegetables + salads]

Starch = Σ [cereals+ rice+ pasta]

Meat= Σ [red meat+ white meat]

Fish = Σ [fatty + lean fish]

Fats (grams/day)= Σ [margarine + olive oil (grams/day)]

Savoury snacks= Σ [salty snacks + pies]

Macronutrient composition baseline (% of daily energy intake) per group:

Intervention group: CHO: 39.2%, Protein: 16.6%, Fat: 44.2%

Control group: CHO: 38.5%, Protein: 15.6%, Fat: 50.4%

Table 13 shows at baseline the daily energy intake for both groups exceeded the recommended estimated average requirements for children aged 5-12 years old [1545 kcal-1845 kcal (females); 1715 kcal-2220 kcals (males)] according to the Hellenic Dietary guidelines for children and adolescents⁽²⁶⁸⁾. From the composition of macronutrients intake for both groups, it is apparent that children's eating patterns are not consistent with the guidelines (50% CHO, 20% P, 30% Fat)⁽²⁶⁹⁾. Children's diets were low in cereals, pasta, rice, fruits, legumes, vegetables (< 400 g/day), average in protein (< 20%) but high in fat (> 30-35%)⁽²⁶⁸⁾ which is consistent with the low KIDMED score obtained from both groups. In other words, it seems that Greek asthmatic children have low adherence to the Mediterranean diet and are adopting a Western-type of eating pattern. On the other hand, fibre intake was within the recommended range of 20-25 g/day⁽³⁰⁷⁾. Nevertheless the macronutrient profile was close to the Traditional Mediterranean diet of 40% carbohydrates, 20% protein and 40% fat⁽²⁶⁹⁾. As for water intake, hydration was low in the intervention group (< 2 litres/day) and above 2 litres in the control group, probably due to the high intake of chocolate milk which would contribute to the water content of the diet.

Table 14. Fatty acid composition of main food groups per intervention and control group at baseline

FATTY ACID ANALYSIS OF FOOD GROUPS							
Baseline							
FOOD GROUPS (grams/day)	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	TRANS (g)	Cholesterol (mg)
Dairy products							
Intervention: 486.91	116.04	222.03	6.82			3.89	84.24
Control: 513.21	173.00	234.00	7.18			4.10	88.79
Fruit							
Intervention: 282.33	Tr	Tr	0.28			-	0.00
Control: 251.94	Tr	Tr	0.25			-	0.00
Vegetables							
Intervention: 167.74	2.57	10.52	3.24			0.00	0.00
Control: 174.68	2.67	10.95	3.37			0.00	0.00
Legumes							
Intervention: 65.49	0.77	2.59	0.64			0.00	2.84
Control: 56.07	0.66	2.23	0.55			0.00	2.42
Starch							
Intervention: 113.47	1.07	1.07	1.95			Tr	0.00
Control: 67.02	0.65	0.63	1.15			Tr	0.00
Meat							
Intervention: 84.03	19.60	16.69	2.41			2.88	164.14
Control: 81.04	18.91	16.10	2.33			2.78	158.29
Seafood							
Intervention: 7.02	0.16	0.42	0.10			0.00	11.58
Control: 4.65	0.11	0.28	0.07			0.00	7.67
Fish							
Intervention: 34.72	1.14	2.67	1.43	0.22	0.42	0.00	46.0
Control: 22.12	0.72	1.70	0.91	0.14	0.26	0.00	29.31
Fats							
Intervention: 20.28	7.49	9.77	1.37			2.47	57.79
Control: 25.87	9.56	12.47	1.75			3.16	73.73
Fast foods							
Intervention: 19.73	0.61	0.69	0.22			0.04	4.80
Control: 26.81	0.82	0.93	0.30			0.05	6.52
Sweets							
Intervention: 25.82	2.29	1.28	0.28			0.16	5.16
Control: 27.89	2.47	1.38	0.29			0.17	5.58
Savoury snacks							
Intervention: 27.22	5.27	6.28	1.84			-	17.42
Control: 43.97	8.51	10.15	2.96			-	28.14
Total intake (units/day)							
Intervention	157.01	274.01	20.58	0.22	0.42	9.44	393.97
Control	218.08	290.82	21.11	0.14	0.26	10.26	400.45
Tr-Trace							

Total fat intake: Intervention group: 471.12 grams/day; Control group: 540.67 grams/day

% Saturated fatty acids of Energy Intake (EI):

Intervention group: $157.01 \times 9 / 3006.60 \times 100\% = 47.03\%$

Control group: $218.08 \times 9 / 2902.04 \times 100\% = 67.63\%$

% MUFA intake of EI:

Intervention: $274.01 \times 9 / 3006.60 \times 100\% = 55.47\%$

Control: $290.82 \times 9 / 2902.04 \times 100\% = 90.2\%$

% PUFA intake:

Intervention: $20.58 \times 9 / 3006.60 \times 100\% = 6.1\%$

Control: $21.11 \times 9 / 2902.04 \times 100\% = 6.5\%$

% Trans fatty acid intake:

Intervention; $9.44 \times 9 / 3006.6 \times 100\% = 2.82\%$

Control; $10.26 \times 9 / 2902.04 \times 100\% = 3.18\%$

At baseline with respect to children's daily intake of fatty acids, Table 14 shows that for both groups percentage of saturated fatty acid and MUFA intake exceeded the recommended dietary reference values of 11% and 13% of daily total energy intake and PUFA intake was about 6.5% as recommended ⁽³⁰⁷⁾. As for the intake of omega 3 fatty acids (EPA/DHA), children in the intervention group consumed 0.60 g/day of EPA and DHA, whereas the control group only 0.40 g/d which does not meet current guidelines. The Hellenic dietary guidelines for children and adolescents recommend at least two fish meals per week (150 g) ⁽²⁶⁸⁾ with one meal containing fatty fish. According to the Scientific Advisory Committee on Nutrition (SACN), two fish meals per week provide 450 mg of omega-3 fatty acids EPA/DHA per day (0.45 g/day) ⁽²⁸⁷⁾. SACN emphasized that this recommendation represents a minimal and achievable average population goal and does not correspond to the level of fish consumption required for maximum nutritional benefit. In other words, with higher intakes more health benefits would be expected. As for dietary cholesterol, both groups were above the recommended intake of 300 mg/day ⁽³⁰⁸⁾

Table 15. Micronutrient analysis of main food groups per intervention and control group at baseline

NUTRIENT ANALYSIS OF FOOD GROUPS (MINERALS)														
BASELINE														
FOOD GROUP (grams/day)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β-Carotene (µg)
Dairy Products														
Intervention: 486.91	128.54	4017	5293.49	501.52	5638.41	6.09	0.63	26.73	20884.52	0.49	73.04	516.12	2380.98	1285.44
Control: 513.20	135.49	4233.98	5583.72	528.60	5942.87	6.41	0.67	28.17	22011	0.51	76.98	544.00	2509.59	1354.87
Fruit														
Intervention: 282.33	28.70	874.29	76.23	59.77	73.88	1.02	0.17	0.42	95.99	1.41	5.65	14.96	0.00	99.29
Control: 251.94	25.62	780.18	68.02	53.33	65.93	0.91	0.15	0.38	85.66	1.26	5.04	13.35	0.00	88.61
Vegetables														
Intervention: 167.74	110.25	1288.8	218.4	87.68	111.16	3.72	0.22	1.46	103.12	1.24	5.03	7.05	0.00	8167.4
Control: 174.68	114.80	1342.11	227.43	91.30	115.76	3.88	0.23	1.52	107.39	1.29	5.24	7.34	0.00	8505.29
Legumes														
Intervention: 65.49	8.02	0.05	23.45	27.7	61.76	1.37	0.20	0.69	8.10	0.33	7.53	3.93	0.00	48.79
Control: 56.07	6.87	184.08	20.07	23.72	52.87	1.18	0.17	0.59	6.94	0.28	6.45	3.36	0.00	41.77
Starch														
Intervention: 113.47	692.46	475.52	147.08	117.8	410.15	63.05	0.88	3.54	1288.34	1.589	26.67	54.47	0.00	0.00
Control: 67.02	409.22	280.81	86.86	69.57	242.21	37.24	0.52	2.09	760.81	0.94	15.75	32.17	0.00	0.00
Meat														
Intervention: 84.03	123.19	490.18	27.99	36.41	285.67	2.04	0.53	4.03	119.59	0.02	16.23	13.17	22.69	Tr
Control: 81.04	118.80	472.74	26.99	35.11	275.11	1.97	0.51	3.89	115.34	0.02	15.65	12.69	21.88	Tr
Seafood														
Intervention: 7.02	26.96	22.16	5.36	1.61	16.82	0.04	0.02	0.13	61.19	0.00	2.03	1.72	2.06	0.91
Control: 4.65	19.67	14.68	3.55	1.07	11.15	0.03	0.02	0.09	40.53	0.00	1.34	1.14	1.36	0.60
Fish														
Intervention: 34.72	94.14	256.40	56.4	21.25	193.6	0.49	0.04	0.37	168.64	0.02	27.43	88.97	11.53	Tr
Control: 22.12	59.98	163.36	35.93	13.54	123.34	0.31	0.03	0.24	107.44	0.013	17.47	56.68	7.34	Tr

Fats														
Intervention: 20.28	190.63	1.01	0.81	0.2	2.43	0.07	0.01	Tr	243.36	Tr	Tr	Tr	134.86	152.1
Control: 25.87	243.18	1.29	1.03	0.26	3.1	0.09	0.01	Tr	310.44	Tr	Tr	Tr	172.04	194.03
Fast foods														
Intervention: 19.73	76.4	35.45	61.95	4.54	27.95	0.02	0.02	1.02	106.9	0.05	2.27	0.47	6.60	20.22
Control: 26.81	103.84	48.18	84.18	6.17	37.98	0.34	0.03	1.39	145.31	0.07	3.08	0.64	8.98	27.48
Sweets														
Intervention: 25.82	48.71	45.99	23.8	5.51	35.29	0.27	0.03	0.15	94.24	0.08	1.16	6.2	22.97	7.44
Control: 27.89	52.62	49.64	25.7	5.95	38.12	0.29	0.03	0.16	101.79	0.08	1.26	6.69	24.82	8.03
Savoury snacks														
Intervention: 27.22	316.3	350.04	56.21	22.32	66.96	0.73	0.04	0.37	356.6	0.10	0.27	N	21.78	247.43
Control: 43.97	510.93	565.58	90.79	36.06	108.17	1.19	0.06	0.06	576.00	0.16	0.44	N	35.18	399.69
<hr/>														
Total intake(units/day):														
Intervention	1844.30	7856.90	5990.68	886.31	6924.08	78.91	2.79	33.91	23530.59	5.33	167.31	707.06	2603.47	10029.02
Control	1801.02	8136.63	6254.27	864.68	7016.61	53.84	2.43	38.58	24368.65	4.62	148.70	678.06	2731.19	10620.37
<hr/>														
RNI (4-14 years)	700-1600 ^a	1100-3100	450-1000	120-280	350-775	6.1-11.3	0.6-0.8	6.5-9.0	1100-2500	16µg	20-45	100-130	*	*

*RNI- Reference Nutrient Intakes according to Department of Health, UK (2010) ⁽³⁰⁷⁾

^a -WHO recommendation for Sodium <2 g Na/day or 5 g salt/day for children ⁽³⁰⁹⁾

*Vitamin A: 400-600 µg/day ⁽³⁰⁷⁾

Table 15 shows that at baseline for both groups micronutrient composition exceeds the daily RNI values, although according to WHO guidelines for sodium intake in adults and children sodium consumption should be below the recommended 2 g Na/day which is the equivalent to one teaspoon (5g) of table salt per day. WHO recommends a reduction in sodium intake (< 2 g Na/day) in an effort to control blood pressure in children ⁽³⁰⁹⁾.

Intervention: 34.72	2.61	0.64	0.09	0.12	3.89	2.87	0.30	3.64	8.61	0.51	3.21	Tr
Control: 22.12	1.67	0.41	0.06	0.08	2.49	1.83	0.19	2.32	5.48	0.32	2.05	Tr
Fats												
Intervention: 20.28	1.6	0.90	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 25.87	2.04	1.15	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Fast foods												
Intervention: 19.73	0.06	0.16	0.04	0.02	0.37	0.47	0.02	0.17	3.10	0.09	0.49	0.39
Control: 26.81	0.08	0.22	0.05	0.03	0.51	0.64	0.27	0.23	4.16	0.12	0.67	0.54
Sweets												
Intervention: 25.82	0.09	0.31	0.02	0.05	0.16	0.013	0.013	0.15	5.53	0.16	1.11	0.26
Control: 27.89	0.09	0.33	0.03	0.06	0.18	0.37	0.01	0.17	5.97	0.18	1.19	0.28
Savoury snacks												
Intervention: 27.22	0.00	1.97	0.09	0.07	0.87	0.35	0.25	0.00	0.03	0.25	N	11.7
Control: 43.97	0.00	3.19	0.14	0.11	0.14	0.57	0.41	0.00	13.18	0.41	N	18.91
Total Intake												
(units/ day)												
Intervention	9.84	20.00	2.84	7.78	32.51	78.14	6.88	31.09	1076.62	17.04	82.75	495.14
Control	9.15	15.92	2.62	7.76	25.73	79.25	5.31	30.07	1014.19	16.96	81.13	463.28
*RNI (4-14 years)	10µg	0.4mg/g PUFA	0.7-0.9	0.8-1.2	11-15	12mg/Kgbwt ^a	0.9-1.2	0.8-1.2	100-200	0.2 ^b	0.9 ^b	30-35

Key: Tr-Trace; N-Nil

*RNI- Reference Nutrient Intakes according to Department of Health, UK (2016) ⁽³⁰⁷⁾; a ⁽³¹⁰⁾ ; b ⁽³¹¹⁾

At baseline, Table 16 illustrates that children in both groups exceeded the daily recommended nutrient intake values for vitamins C, E, B and folate, except for vitamin D (< 10 µg).

Nutrient composition at six-months

Nutrient analysis of food frequency questionnaires at the six-month follow-up are presented in Table 17 to Table 24.

Table 17. Macronutrients analysis of dietary intake for intervention and control group at six-months

NUTRIENT ANALYSIS (MACRONUTRIENTS)						
Six –Months						
Food item/Group (grams/day)	H ₂ O (g)	CHO (g)	P (g)	FAT (g)	Energy (kcal)	Fibre (g)
Milk						
Intervention: 331.28	290.20	15.90	10.60	11.20	205.39	0.00
Control: 363.05	318.03	17.43	11.62	12.34	225.09	0.00
Chocolate milk						
Inter: 47.34	39.20	4.54	1.70	0.71	29.82	0.00
Control: 74.51	61.69	7.15	2.68	1.12	46.94	0.00
Yogurt						
Intervention: 54.10	44.31	4.22	3.08	1.62	42.74	0.00
Control: 61.91	50.70	4.83	3.53	1.86	48.91	0.00
Cheese						
Intervention: 20.37	20.43	0.59	8.61	9.41	120.39	0.00
Control: 17.76	17.81	0.51	7.51	8.20	104.96	0.00
Fruit						
Intervention: 142.62	118.37	24.25	1.33	0.29	92.66	2.09
Control: 117.38	97.43	19.95	1.09	0.23	76.26	1.72
Fruit juice						
Intervention: 135.59	121.08	12.61	0.41	0.13	49.49	0.13
Control: 137.15	166.10	17.29	0.56	0.18	67.88	0.18
Vegetables stewed						
Intervention: 41.20	32.05	2.58	0.77	3.78	46.14	1.19
Control: 37.68	29.31	2.36	0.70	3.46	42.20	1.08
Salads						
Intervention: 99	92.07	3.72	0.91	0.26	18.81	1.39
Control: 140.80	130.94	5.29	1.29	0.37	26.75	1.97
Vegetables boiled						
Intervention: 32.38	29.08	1.04	0.81	0.19	8.80	0.59
Control: 27.75	24.91	0.86	0.69	0.17	7.55	0.50
Legumes						
Intervention: 59.81	31.45	7.50	3.04	4.99	76.44	1.87
Control: 46.81	24.61	5.87	2.38	3.91	59.82	1.46
Cereals						
Intervention: 27.52	4.91	17.98	2.56	0.55	84.16	1.78
Control: 23.35	4.17	15.25	2.17	0.47	71.40	1.51
Pasta						
Intervention: 36.34	23.35	11.55	2.39	0.55	57.78	0.69
Control: 32.05	19.70	10.19	2.11	0.48	50.96	0.61

NUTRIENT ANALYSIS (MACRONUTRIENTS)

Six –Months

Food item/Group (grams/day)	H ₂ O (g)	CHO (g)	P (g)	FAT (g)	Energy (kcal)	Fibre (g)
Rice						
Intervention: 30.32	20.61	9.37	0.79	0.39	41.84	0.03
Control: 20.38	13.85	6.29	0.53	0.26	28.12	0.02
Red meat						
Intervention: 40.08	14.12	0.00	8.01	18.10	195.06	0.00
Control: 50.35	17.74	0.00	10.07	22.74	245.04	0.00
White meat						
Intervention: 29.52	20.74	0.00	7.03	1.24	39.26	0.00
Control: 32.52	22.85	0.00	7.75	1.37	43.25	0.00
Seafood						
Intervention: 5.60	3.57	0.49	1.17	0.52	10.77	0.03
Control: 7.09	4.53	0.63	1.48	0.65	13.63	0.03
Lean fish						
Intervention: 15.77	12.11	0.00	3.38	0.31	16.36	0.00
Control: 13.20	10.14	0.00	2.83	0.26	13.69	0.00
Fatty fish						
Intervention: 45.76	27.93	0.00	10.36	5.07	83.92	0.00
Control: 10.35	6.31	0.00	2.34	1.15	18.98	0.00
Traditional meals						
Intervention: 19.06	12.98	2.11	1.42	2.00	31.58	0.23
Control: 16.68	11.36	18.46	1.25	1.75	27.64	0.20
Margarine						
Intervention: 2.13	0.00	0.00	0.00	1.76	15.34	0.00
Control: 1.46	0.00	0.00	0.00	1.21	10.51	0.00
Nuts						
Intervention: 3.81	3.46	0.37	0.78	2.14	24.18	0.18
Control: 4.45	0.12	0.44	0.91	2.49	28.25	0.21
Olive oil						
Intervention: 18.08	Tr	0.00	0.00	17.89	162.54	0.00
Control: 18.98	Tr	0.00	0.00	18.70	170.63	0.00
Fast foods						
Intervention: 19.61	9.37	6.06	2.38	1.63	47.13	0.35
Control: 23.75	11.35	7.34	2.88	1.98	57.08	0.42
Pies						
Intervention: 19.95	11.03	3.28	1.59	3.55	50.37	0.32
Control: 26.71	14.77	4.39	2.14	4.75	67.44	0.43
Sweets						
Intervention: 20.58	6.29	9.86	1.2	4.65	75.16	0.27
Control: 29.89	7.49	14.32	1.75	5.43	109.16	0.39
Salty snacks						
Intervention: 8.98	0.25	4.79	0.51	3.07	47.59	0.48
Control: 12.55	0.35	6.69	0.72	4.29	66.52	0.67
Soft drinks						
Intervention: 36.14	32.09	3.90	Tr	0.00	14.64	0.00
Control: 36.02	31.99	3.89	Tr	0.00	14.59	0.00

Table 18. Fatty acid composition of dietary intake for intervention and control group at six months

NUTRIENT ANALYSIS (FATTY ACID COMPOSITION)							
Six-months							
FOOD ITEM (grams/day)	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	TRANS (g)	Cholesterol (mg)
Milk							
Intervention: 331.28	7.29	3.31	0.33			0.33	46.38
Control: 363.05	7.99	3.63	0.36			0.36	50.83
Chocolate milk							
Inter: 47.34	0.47	0.14	0.05			Tr	3.31
Control: 74.51	0.75	0.22	0.07			Tr	5.21
Yogurt							
Intervention: 54.10	0.92	0.49	0.11			N	0.54
Control: 61.91	1.05	0.56	0.12			N	6.81
Cheese							
Intervention: 20.37	6.00	1.89	0.20			0.14	28.72
Control: 17.76	5.24	1.65	0.18			0.12	25.04
Fruit							
Intervention: 142.62	0.14	Tr	0.14			-	0.00
Control: 117.38	0.12	Tr	0.12			-	0.00
Fruit juice							
Intervention: 135.59	Tr	Tr	Tr			-	0.00
Control: 137.15	Tr	Tr	Tr			-	0.00
Vegetables stewed							
Intervention: 41.20	0.55	2.50	0.49			-	0.00
Control: 37.68	0.50	2.29	0.45			-	0.00
Salads							
Intervention: 99	0.10	0.10	0.23			0.00	0.00
Control: 140.80	0.14	0.14	0.32			0.00	0.00
Vegetables boiled							
Intervention: 32.38	0.05	0.03	0.11			0.00	0.00
Control: 27.75	0.04	0.03	0.09			0.00	0.00
Legumes							
Intervention: 59.81	0.71	2.37	0.59			0.00	2.59
Control: 46.81	0.55	1.86	0.46			0.00	2.03
Cereals							
Intervention: 27.52	0.09	0.094	0.23			Tr	0.00
Control: 23.35	0.08	0.08	0.19			Tr	0.00
Pasta							
Intervention: 36.34	0.11	0.10	0.14			-	N
Control: 32.05	0.10	0.10	0.09			-	N
Rice							
Intervention: 30.32	0.09	0.09	0.15			0.00	0.00
Control: 20.38	0.06	0.06	0.10			0.00	0.00
Red meat							
Intervention: 40.08	8.75	4.37	0.76			1.33	41.68
Control: 50.35	10.99	9.26	0.96			1.68	52.36
White meat							
Intervention: 29.52	0.44	0.43	0.29			0.03	26.96
Control: 32.52	0.49	0.48	0.31			0.03	32.44

NUTRIENT ANALYSIS (FATTY ACID COMPOSITION)							
Six-months							
FOOD ITEM (grams/day)	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	TRANS (g)	Cholesterol (mg)
Seafood							
Intervention: 5.60	0.13	0.33	0.08			0.00	9.24
Control: 7.09	0.16	0.42	0.106			0.00	11.69
Lean fish							
Intervention: 15.77	0.06	0.10	0.10	0.02	0.02	0.00	8.95
Control: 13.20	0.05	0.08	0.09	0.01	0.03	0.00	7.49
Fatty fish							
Intervention: 45.76	1.32	3.23	1.59	0.25	0.44	0.00	34.66
Control: 10.35	0.29	0.73	0.36	0.06	0.10	0.00	7.48
Traditional meals							
Intervention: 19.06	0.63	0.99	0.22			0.08	4.765
Control: 16.68	0.55	0.87	0.19			0.07	4.17
Margarine							
Intervention: 2.13	0.74	0.77	0.12			0.26	6.07
Control: 1.46	0.50	0.53	0.08			0.18	4.16
Nuts							
Intervention: 3.81	0.28	0.98	0.79			0.00	0.00
Control: 4.45	0.32	1.14	0.92			0.00	0.00
Olive oil							
Intervention: 18.08	2.59	13.02	1.48			0.00	0.00
Control: 18.98	2.71	13.85	1.56			0.00	0.00
Fast foods							
Intervention: 19.61	0.60	0.69	0.22			0.04	4.77
Control: 23.75	0.73	0.83	0.27			0.05	5.78
Pies							
Intervention: 19.95	1.07	1.88	0.35			-	12.77
Control: 26.71	1.43	2.51	0.47			-	17.09
Sweets							
Intervention: 20.58	1.21	1.02	0.22			0.13	4.10
Control: 29.89	2.65	1.48	0.32			0.19	5.98
Salty snacks							
Intervention: 8.98	1.26	1.23	0.45			N	0.00
Control: 12.55	1.76	1.72	0.63			N	0.00
Soft drinks							
Intervention: 36.14	0.00	0.00	0.00			0.00	0.00
Control: 36.02	0.00	0.00	0.00			0.00	0.00

Key: Tr-Trace; N-Nil

NUTRIENT ANALYSIS (MINERALS)

SIX MONTHS

FOOD ITEM	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β- Carotene (µg)
Intervention: 99.00	8.70	196.00	23.96	6.14	29.10	0.44	0.01	0.14	34.80	0.16	0.99	2.18	0.00	2830.6
Control: 140.80	12.39	278.8	34.07	8.73	41.39	0.619	0.02	0.20	49.56	0.22	1.40	3.10	0.00	4025.7
Vegetables boiled														
Intervention: 32.38	16.06	80.30	17.14	5.96	16.82	0.28	0.01	0.13	6.89	0.12	0.32	0.65	0.00	492.56
Control: 27.75	13.76	68.82	14.7	5.10	14.43	0.24	0.00	0.11	5.90	0.10	0.28	0.56	0.00	422.13
Legumes														
Intervention: 59.81	7.33	196.32	21.41	25.29	56.39	1.25	0.18	0.62	7.40	0.29	6.88	3.59	0.00	44.55
Control: 46.81	5.73	153.67	16.76	19.80	44.14	0.98	0.15	0.49	5.79	0.23	5.38	2.81	0.00	34.87
Cereals														
Intervention: 27.52	163.36	86.96	20.53	20.31	60.93	15.01	0.05	0.45	303.32	0.22	1.51	1.93	0.00	0.00
Control: 23.35	138.6	73.79	17.42	17.23	51.69	12.74	0.04	0.38	257.40	0.19	1.28	1.63	0.00	0.00
Pasta														
Intervention: 36.34	5.81	17.81	13.45	6.90	31.25	0.29	0.16	0.29	10.54	0.14	4.72	13.08	0.00	0.00
Control: 32.05	5.13	15.70	11.86	6.09	27.56	0.26	0.15	0.26	9.29	0.13	4.17	11.54	0.00	0.00
Rice														
Intervention: 30.32	0.30	16.37	5.46	3.34	16.37	0.06	0.04	0.21	1.20	0.06	1.50	1.50	0.00	0.00
Control: 20.38	0.20	11.00	3.67	2.24	11.00	0.04	0.03	0.14	0.81	0.04	1.00	1.00	0.00	0.00
Red Meat														
Intervention: 40.08	25.65	110.89	4.94	7.61	66.80	0.56	0.23	1.01	24.85	0.00	1.47	3.47	2.80	Tr
Control: 50.35	32.22	139.30	6.21	9.56	83.92	0.70	0.29	1.27	31.22	0.00	1.85	4.36	3.52	Tr
White meat														
Intervention: 29.52	24.38	90.53	6.20	6.59	51.16	0.30	0.02	0.67	23.71	0.00	4.63	2.07	5.90	Tr
Control: 32.52	26.86	99.73	6.83	7.26	0.05	0.33	0.02	0.74	26.12	0.00	5.09	2.28	6.50	Tr

NUTRIENT ANALYSIS (MINERALS)														
SIX MONTHS														
FOOD ITEM	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β- Carotene (µg)
Seafood														
Intervention: 5.60	23.69	17.67	4.27	1.29	13.42	0.03	0.02	0.10	48.82	0.00	1.62	1.37	1.64	0.73
Control: 7.09	29.99	22.38	5.41	1.63	16.99	0.04	0.02	0.13	61.8	0.00	2.05	1.74	2.08	0.92
Lean fish														
Intervention: 15.77	27.72	57.17	2.48	4.10	38.64	0.06	0.00	0.08	35.88	0.00	7.09	30.75	0.32	Tr
Control: 13.20	23.19	47.85	2.08	3.43	32.34	0.05	0.00	0.07	30.08	0.00	5.94	25.74	0.26	Tr
Fatty fish														
Intervention: 45.76	43.66	172.06	67.13	16.11	143.06	0.48	0.05	0.39	118.15	0.02	14.19	28.02	14.28	Tr
Control: 10.35	9.87	38.91	15.15	3.64	32.36	0.11	0.01	0.09	26.72	0.00	3.21	6.34	3.23	Tr
Traditional meals														
Intervention: 19.06	31.01	45.11	12.33	6.86	19.57	0.14	0.02	0.16	-	0.02	N	N	9.9	54
Control: 16.68	27.14	39.48	10.79	6.00	0.85	0.13	0.05	0.14	-	0.02	N	N	8.67	47.26
Margarine														
Intervention: 2.13	20.02	0.11	0.09	0.02	0.25	0.01	0.00	N	25.56	Tr	Tr	N	14.16	15.98
Control: 1.46	13.72	0.07	0.06	0.01	0.18	0.00	0.00	N	17.52	Tr	Tr	N	9.71	10.95
Nuts														
Intervention: 3.81	10.49	25.62	4.01	8.29	17.72	0.14	0.05	0.14	15.93	0.09	0.4	0.39	0.00	0.00
Control: 4.45	12.25	29.92	4.68	9.68	20.69	0.16	0.06	0.17	18.60	0.10	0.47	0.46	0.00	0.00
Olive oil														
Intervention: 18.08	Tr	Tr	Tr	Tr	Tr	0.01	0.00	Tr	Tr	Tr	Tr	Tr	0.00	N
Control: 18.98	Tr	Tr	Tr	Tr	Tr	0.01	0.00	Tr	Tr	Tr	Tr	Tr	0.00	N
Fast foods														
Intervention: 19.61	75.95	39.16	61.57	4.51	27.78	0.25	0.02	1.02	106.30	0.05	2.26	4.71	6.57	20.10
Control: 23.75	91.98	47.43	74.58	5.46	33.65	0.30	0.03	1.23	128.72	0.06	2.73	5.70	7.95	24.34

Pies														
Intervention: 19.95	72.22	45.08	35.41	4.99	27.13	0.26	-	0.15	-	-	-	-	15.96	180.95
Control: 26.71	96.69	60.36	47.41	6.65	36.33	0.35	-	0.20	-	-	-	-	21.37	242.26
Sweets														
Intervention: 20.58	38.77	36.63	18.96	4.39	28.11	0.22	0.02	0.12	75.12	0.062	0.93	4.94	18.32	5.93
Control: 29.89	56.39	53.20	27.55	6.38	40.83	0.32	0.03	0.17	109.10	0.09	1.35	7.17	26.60	8.61
Salty snacks														
Intervention: 8.98	71.84	95.19	2.60	5.12	9.88	0.12	0.01	0.05	117.64	0.03	0.09	N	0.00	0.18
Control: 12.55	100.4	133.03	3.64	7.15	13.80	0.17	0.02	0.07	164.4	0.05	0.13	N	0.00	0.25
Soft drinks														
Intervention: 36.14	4.16	4.52	1.89	0.90	3.97	Tr	Tr	Tr	2.28	Tr	Tr	Tr	0.00	167.87
Control: 36.02	4.14	4.50	1.89	0.90	3.96	Tr	Tr	Tr	2.27	Tr	Tr	Tr	0.00	167.31

Key: Tr-Trace; N-Nil

Table 20. Vitamin composition of dietary data for intervention and control group at six months

NUTRIENT ANALYSIS (VITAMINS)												
SIX MONTHS												
FOOD ITEM (grams/day)	Vitamin D (µg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tryptophan (mg)	B6 (mg)	B12 (µg)	Folate (µg)	Pantothenate (mg)	Biotin (µg)	Vitamin C (mg)
Milk												
Intervention: 331.28	Tr	0.27	0.10	0.76	0.66	1.99	0.20	2.98	26.5	1.92	8.28	6.63
Control: 363.05	Tr	0.29	0.11	0.84	0.73	2.18	0.22	3.27	29.04	2.10	9.07	7.26
Chocolate milk												
Intervention: 47.34	0.00	0.01	0.01	0.08	0.05	0.38	0.01	0.05	0.95	0.14	1.04	Tr
Control: 74.51	0.00	0.02	0.02	0.02	0.07	0.60	0.02	0.07	1.49	0.22	1.64	Tr
Yogurt												
Intervention: 54.10	0.00	0.03	0.03	0.15	0.11	0.70	0.05	0.11	9.74	0.27	1.41	0.54
Control: 61.91	0.00	0.03	0.04	0.17	0.12	0.80	0.06	0.12	11.14	0.31	1.61	0.62
Cheese												
Intervention: 20.37	0.16	0.24	0.01	0.11	0.06	1.96	0.03	0.65	12.63	0.15	0.86	Tr
Control: 17.76	0.14	0.21	0.01	0.10	0.05	1.70	0.03	0.57	11.01	0.13	0.75	Tr
Fruit												
Intervention: 142.62	0.00	0.37	0.089	0.057	0.57	0.19	0.21	0.00	23.77	0.34	2.28	37.51
Control: 117.38	0.00	0.31	0.074	0.047	0.47	0.15	0.18	0.00	19.57	0.28	1.88	30.87
Fruit juice												
Intervention: 135.59	0.00	0.11	0.05	0.01	0.02	0.13	0.05	0.00	13.56	0.09	1.36	43.39
Control: 137.15	0.00	0.12	0.05	0.01	0.02	0.14	0.05	0.00	13.72	0.09	1.37	43.89
Vegetables stewed												
Intervention: 41.20	0.00	0.75	0.06	0.01	0.37	0.18	0.09	0.00	18.13	0.10	0.20	9.34
Control: 37.68	0.00	0.68	0.05	0.01	0.34	0.17	0.08	0.00	16.58	0.09	0.18	8.54
Salads												
Intervention: 99.00	0.00	0.51	0.07	0.02	0.63	0.21	0.10	0.00	33.80	0.24	0.75	15.20

Control: 140.80	0.00	0.73	0.11	0.028	0.9	0.31	0.15	0.00	48.15	0.34	1.07	21.68
Vegetables boiled												
Intervention: 32.38	0.00	0.24	0.02	0.01	0.15	0.17	0.06	0.04	21.82	0.07	0.18	30.76
Control: 27.75	0.00	0.20	0.01	0.01	0.13	0.14	0.05	0.03	18.70	0.06	0.15	26.36
Legumes												
Intervention: 59.81	0.00	0.46	0.10	0.04	1.05	1.00	0.10	0.00	41.45	1.23	1.25	2.69
Control: 46.81	0.00	0.38	0.01	0.03	0.82	0.79	0.08	0.00	32.44	0.96	0.98	2.11
Cereals												
Intervention: 27.52	0.23	0.65	0.15	0.15	2.31	0.52	0.25	0.36	51.8	0.15	1.38	18.16
Control: 23.35	0.20	0.55	0.13	0.12	1.96	0.44	0.21	0.30	43.96	0.13	1.17	15.41
Pasta												
Intervention: 36.34	0.00	Tr	0.02	0.01	0.25	0.36	0.01	0.00	1.45	Tr	Tr	0.00
Control: 32.05	0.00	Tr	0.02	0.01	0.22	0.32	0.01	0.00	1.28	Tr	Tr	0.00
Rice												
Intervention: 30.32	0.00	Tr	0.00	Tr	0.27	0.18	0.02	0.00	2.12	0.03	0.30	0.61
Control: 20.38	0.00	Tr	0.00	Tr	0.18	0.12	0.01	0.00	1.43	0.02	0.20	0.40
Red meat												
Intervention: 40.08	0.22	0.06	0.04	0.09	1.49	1.27	0.09	0.80	4.68	0.36	0.67	0.00
Control: 50.35	0.28	0.08	0.05	0.11	1.88	1.59	0.12	1.00	5.86	0.45	0.84	0.00
White meat												
Intervention: 29.52	0.06	0.03	0.02	0.06	1.87	1.47	0.1	0.59	3.35	0.29	0.69	0.00
Control: 32.52	0.02	0.01	0.01	0.02	0.61	0.48	0.03	0.19	1.09	0.09	0.22	0.00
Seafood												
Intervention: 5.60	0.01	0.02	0.01	0.17	0.05	0.20	0.01	0.56	0.84	0.02	0.06	0.28
Control: 7.09	0.01	0.03	0.01	0.21	0.07	0.26	0.02	0.71	1.06	0.02	0.07	0.35
Lean fish												
Intervention: 15.77	Tr	0.09	0.01	0.02	0.79	0.65	0.06	0.39	1.97	0.05	0.47	Tr
Control: 13.20	Tr	0.07	0.01	0.02	0.66	0.55	0.05	0.33	1.65	0.04	0.39	Tr
Fatty fish												
Intervention: 45.76	3.45	0.57	0.08	0.11	2.86	1.89	0.21	3.66	5.63	0.52	2.86	Tr

Control: 10.35	0.78	0.13	0.02	0.02	0.65	0.43	0.05	0.83	1.27	0.12	0.65	Tr
Traditional meals												
Intervention: 19.06	0.06	0.19	0.01	0.02	0.28	0.28	0.03	0.19	1.52	0.09	0.38	3.53
Control: 16.68	0.05	0.17	0.01	0.02	0.25	0.25	0.03	0.17	1.33	0.04	0.33	3.09
Margarine												
Intervention: 2.13	0.17	0.09	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 1.46	0.12	0.06	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Nuts												
Intervention: 3.81	0.00	0.29	0.01	0.01	0.18	0.16	0.02	0.00	2.15	0.04	2.15	0.00
Control: 4.45	0.00	0.34	0.01	0.01	0.21	0.19	0.02	0.00	2.51	0.05	2.51	0.00
Olive oil												
Intervention: 18.08	0.00	0.92	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 18.98	0.00	0.97	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Fast foods												
Intervention: 19.61	0.06	0.16	0.03	0.02	0.37	0.47	0.02	0.17	3.04	0.09	0.49	0.39
Control: 23.75	0.07	0.19	0.04	0.03	0.45	0.57	0.02	0.20	3.68	0.10	0.59	0.47
Pies												
Intervention: 19.95	-	0.25	0.02	0.03	-	-	0.02	-	-	-	-	1.59
Control: 26.71	-	0.33	0.03	0.04	-	-	0.03	-	-	-	-	2.14
Sweets												
Intervention: 20.58	0.07	0.24	0.02	0.04	0.13	0.27	0.01	0.12	4.40	0.13	0.88	0.20
Control: 29.89	0.10	0.36	0.03	0.06	0.19	0.39	0.01	0.18	6.39	0.19	1.28	0.29
Salty snacks												
Intervention: 8.98	0.00	0.54	0.02	0.01	0.29	0.16	0.10	0.00	2.69	0.08	N	3.14
Control: 12.55	0.00	0.75	0.03	0.01	0.40	0.16	0.10	0.00	3.76	0.12	N	4.39
Soft drinks												
Intervention: 36.14	0.00	0.00	Tr	Tr	Tr	Tr	Tr	0.00	0.36	Tr	Tr	1.62
Control: 36.02	0.00	0.00	Tr	Tr	Tr	Tr	Tr	0.00	0.36	Tr	Tr	1.62

Food groups six months analysis

Table 21. Macronutrient composition of main food groups per intervention and control group at six months

NUTRIENT ANALYSIS OF FOOD GROUPS (MACRONUTRIENTS)						
Six-Months						
FOOD GROUP (grams/day)	H ₂ O (g)	CHO (g)	P (g)	Fat (g)	Energy (kcal)	Fibre (g)
Dairy Products						
Intervention 453.09	1598	113.70	248.29	245.12	3602.10	0.00
Control 517.24	1823.79	129.82	283.45	279.83	4112.06	0.00
Fruit						
Intervention: 270.25	465.64	71.08	3.22	0.81	274.22	4.24
Control: 259.08	446.39	68.14	3.09	0.78	262.88	9.06
Vegetables						
Intervention: 172.58	453.20	18.69	8.61	17.65	258.87	10.99
Control: 206.23	541.56	22.33	10.29	21.10	309.34	13.14
Legumes						
Intervention: 59.81	31.45	7.50	3.04	4.99	76.44	1.87
Control: 46.81	24.61	5.87	2.38	3.91	59.82	1.46
Starch						
Intervention: 94.18	138.76	120.53	17.44	4.52	567.78	7.99
Control: 75.78	111.65	97.03	14.03	3.64	456.80	6.43
Meat						
Intervention: 69.60	73.43	5.57	30.50	34.36	431.29	0.0
Control: 82.88	87.44	6.63	36.33	40.92	513.58	0.0
Seafood						
Intervention: 5.60	3.57	0.49	1.17	0.52	10.77	0.03
Control: 7.09	4.53	0.63	1.48	0.65	13.63	0.03
Fish						
Intervention: 61.23	84.43	0.00	26.99	8.00	175.82	0.0
Control: 23.55	32.46	0.00	10.38	3.08	67.62	0.0
Fats						
Intervention: 24.01	0.04	0.00	0.00	43.65	388.72	0.0
Control: 4.88	0.04	0.00	0.00	45.23	402.81	0.0
Fast foods						
Intervention: 19.61	9.37	6.06	2.38	1.63	47.13	0.35
Control: 23.75	11.35	7.34	2.88	1.98	57.08	0.42
Sweets						
Intervention: 20.58	6.29	9.86	1.20	4.65	75.16	0.27
Control: 29.89	7.49	14.32	1.75	5.43	109.16	0.39
Savoury snacks						
Intervention: 28.93	16.81	20.18	3.96	15.04	226.38	1.99
Control: 39.26	22.81	27.38	5.38	20.41	307.21	2.71
Total Intake						
Intervention (grams/day)	2880.99	373.66	346.8	380.94	6134.58	27.73
Control	3114.12	379.49	371.44	426.96	6671.99	28.64

Food groups: fats = margarine + olive oil

Fish group = fatty + lean

Starch = cereals + rice + pasta

Dairy product = milk + chocolate milk + cheese + yogurt

Savoury = salty snacks + pies

Macronutrient composition at six months per total EI per day:

Intervention: % CHO intake $373.66 \times 4 / 6134.68 \times 100\% = 24.4\%$

% Protein intake: $346.8 \times 4 / 6134.8 \times 100\% = 22.6\%$

% Fat intake: $380.94 \times 9 / 6134.8 \times 100\% = 55.9\%$

Intervention group: CHO: 24.4%, P: 22.6%; Fat: 55.9%

Control: % CHO: 22.8%, %P: 22.3%, %Fat: 57.6%

At six months, Table 21 indicates that energy intake in both groups had doubled from baseline and exceeded the recommended EAR of 1545-2220 kcal/day ⁽²⁶⁸⁾. Children in both groups were low in carbohydrate (< 45-60%) and high in fat intake >30-35% as recommended by the Hellenic dietary guidelines for children ⁽²⁶⁸⁾. In fact, fat intake increased in both groups as compared to baseline (baseline vs six-months: Intervention group % fat intake; 44.2% vs 55.9%, control: 50.4% vs 57.6%). In contrast, for both groups protein intake increased by the end of the six month study (% protein intake baseline vs six-months: intervention: 16.6% vs 22.6%, control: 15.6% vs 22.3%) which corresponds to the recommended protein intake according to the Traditional Mediterranean diet of 20% of total daily EI. Fibre intake was within the suggested range of 20-25 g/day ⁽³⁰⁷⁾.

Table 22. Fatty acid composition of main food groups per intervention and control group at six months

FATTY ACID COMPOSITION OF FOOD GROUPS							
Six- Months							
FOOD GROUP	Sat (g)	MUFA (g)	PUFA (g)	EPA (g)	DHA (g)	Trans (g)	Cholesterol (mg)
Dairy Products							
Intervention 453.09	154.50	206.60	6.34			3.62	783.85
Control 517.24	176.38	235.86	7.24			4.14	894.82
Fruit							
Intervention: 270.25	Tr	Tr	0.27			-	0.00
Control: 259.08	Tr	Tr	0.25			-	0.00
Vegetables							
Intervention: 172.58	2.64	10.82	18.86			0.00	0.00
Control: 206.23	3.15	12.93	3.98			0.00	0.00
Legumes							
Intervention: 59.81	0.71	2.37	0.59			0.00	2.59
Control: 46.81	0.55	1.86	0.46			0.00	2.03
Starch							
Intervention: 94.18	0.89	0.89	1.64			Tr	0.00
Control: 75.78	0.71	0.71	1.30			Tr	0.00
Meat							
Intervention: 69.60	16.24	13.83	1.99			2.39	135.95
Control: 82.88	19.32	16.47	2.38			2.83	161.89
Seafood							
Intervention: 5.60	0.13	0.33	0.08			0.00	9.24
Control: 7.09	0.16	0.42	0.11			0.00	11.69
Fish							
Intervention: 61.23	1.98	4.70	2.53	0.39	0.67	0.00	81.13
Control: 23.55	0.77	1.81	0.97	0.15	0.26	0.00	31.20
Fats							
Intervention:24.01	11.74	26.22	3.27			2.93	68.43
Control:4.88	12.17	27.17	3.38			3.04	70.91
Fast foods							
Intervention: 19.61	0.60	0.69	0.22			0.04	4.77
Control: 23.75	0.73	0.83	0.27			0.05	5.78
Sweets							
Intervention: 20.58	1.21	1.02	0.22			0.13	4.10
Control: 29.89	2.65	1.48	0.32			0.19	5.98
Savoury snacks							
Intervention: 28.93	5.59	6.68	1.95			-	18.51
Control: 39.26	7.59	9.07	2.65			-	25.12
Total Intake:							
Intervention group (grams/day)	196.23	274.25	37.96	0.39	0.67	9.11	1108.60
Control group	224.18	308.61	23.31	0.15	0.26	10.25	1209.44

Key: Sat- Saturated fat; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids;

% Saturated fat intake per total daily EI:

Intervention group: $196.23 \times 9 / 6134.6 \times 100\% = 28.8\%$

Control group $224.18 \times 9 / 6672 \times 100\% = 30.24\%$

% MUFA intake:

Intervention; $274.25 \times 9 / 6134.6 \times 100\% = 40.23\%$

Control: $308.61 \times 9 / 6672 \times 100\% = 41.62\%$

% PUFA intake:

Intervention; $37.96 \times 9 / 6134.6 \times 100\% = 5.56$

Control: $23.31 \times 9 / 6672 \times 100\% = 3.14$

% Trans fatty acid intake:

Intervention: $9.11 \times 9 / 6314.6 \times 100\% = 1.34\%$

Control: $10.25 \times 9 / 6672 \times 100\% = 1.38\%$

In Table 22 for both groups at six months the percentage of saturated fat and MUFA intake exceeded the Dietary Reference Values (DRVs) of 11% and 13% respectively. However, PUFA intake and trans fat intake were below the DRVs of 6.5% and 2%. With respect to cholesterol intake, it exceeded the recommended 300 mg/day⁽³⁰⁸⁾.

Table 23 Mineral composition of main food groups for intervention and control group at six months

NUTRIENT ANALYSIS OF FOOD GROUPS (MINERALS)														
Six -Months														
FOOD GROUP	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Cl (mg)	Mn (mg)	Se (µg)	I (µg)	Retinol (µg)	β- Carotene (µg)
Dairy Products														
Intervention 453.09	1178.5	2148.30	4929.62	466.65	5246.78	5.66	0.59	24.87	1912.00	0.45	67.96	480.28	2215.61	1196.16
Control 517.24	1345.36	4266.90	5627.14	532.72	5989.20	6.47	0.67	28.39	2182.70	0.52	77.58	548.23	2529.11	1365.41
Fruit														
Intervention: 270.25	27.48	836.88	72.97	57.21	70.72	0.97	0.15	0.41	91.89	1.35	5.4	14.32	0.00	95.05
Control: 259.08	26.35	1012.15	69.95	54.85	67.80	0.93	0.15	0.39	88.09	1.29	5.18	13.73	0.00	91.12
Vegetables														
Intervention: 172.58	113.44	1325.9	224.69	90.21	114.35	3.83	0.23	1.50	106.1	1.28	5.18	7.25	0.00	8403.04
Control: 206.23	135.55	1584.32	268.51	107.79	136.67	4.58	0.27	1.79	126.8	1.53	6.19	8.67	0.00	10041.48
Legumes														
Intervention: 59.81	7.33	196.32	21.41	25.29	56.39	1.25	0.18	0.62	7.40	0.29	6.88	3.59	0.00	44.55
Control: 46.81	5.73	153.67	16.76	19.80	44.14	0.98	0.15	0.49	5.79	0.23	5.38	2.81	0.00	34.87
Starch														
Intervention: 94.18	575.06	394.60	122.06	97.76	340.37	52.33	0.73	2.94	1069.13	1.32	22.13	45.21	0.00	0.00
Control: 75.78	462.71	317.52	98.21	78.66	273.87	42.10	0.59	2.36	860.25	1.06	17.81	36.37	0.00	0.00
Meat														
Intervention: 69.60	102.03	406.00	23.18	28.27	236.62	1.69	0.44	3.34	99.06	0.02	13.44	10.91	18.79	Tr
Control: 82.88	121.50	483.47	27.58	34.25	281.77	2.01	0.52	3.98	117.96	0.02	16.00	12.98	22.37	Tr
Seafood														
Intervention: 5.60	23.69	17.67	4.27	1.29	13.42	0.03	0.02	0.10	48.82	0.00	1.62	1.37	1.64	0.73
Control: 7.09	29.99	22.38	5.41	1.63	16.99	0.04	0.02	0.13	61.80	0.00	2.05	1.74	2.08	0.92
Fish														
Intervention: 61.23	166.3	452.18	99.47	37.47	341.47	0.92	0.07	0.84	297.40	0.04	46.53	156.9	20.33	Tr
Control: 23.55	63.86	173.55	38.26	14.38	131.33	0.32	0.03	0.32	114.40	0.02	17.89	60.35	7.80	Tr

Fats														
Intervention:24.01	225.70	1.20	0.96	0.24	2.88	0.08	0.01	Tr	288.12	Tr	Tr	Tr	159.67	180.08
Control:4.88	233.87	1.24	0.99	0.25	2.98	0.08	0.01	Tr	298.60	Tr	Tr	Tr	165.45	186.6
Fast foods														
Intervention: 19.61	75.95	39.16	61.57	4.51	27.78	0.25	0.02	1.02	106.30	0.05	2.26	4.71	6.57	20.10
Control: 23.75	91.98	47.43	74.58	5.46	33.65	0.30	0.03	1.23	128.72	0.06	2.73	5.70	7.95	24.34
Sweets														
Intervention: 20.58	38.77	36.63	18.96	4.39	28.11	0.22	0.02	0.12	75.12	0.06	0.93	4.94	18.32	5.93
Control: 29.89	56.39	53.20	27.55	6.38	40.83	0.32	0.03	0.17	109.1	0.09	1.35	7.17	26.6	8.61
Savoury snacks														
Intervention: 28.93	336.17	372.03	59.74	23.72	71.17	0.78	0.04	0.39	378.98	0.11	0.29	N	23.14	262.97
Control: 39.26	456.20	504.88	81.07	32.19	96.58	1.06	0.06	0.53	514.3	0.15	0.39	N	31.41	356.87
Total intake:														
Intervention group (units/d)	2869.80	6226.90	5638.90	837.01	6550.10	68.01	2.51	36.15	4480.62	4.97	172.6	2	729.48	10208.61
Control group (units/d)	3028.63	6336.01	6336.01	888.36	7115.80	59.19	2.53	39.78	4608.51	4.98	152.5	5	697.75	12110.22
*RNI (4-14 years)	700-1600 ^a	1100-3100	450-1000	120-280	350-775	6.1-11.3	0.6-0.8	6.5-9.0	1100-2500	16µg	20-45	100-130	**	**
Key: Tr-Trace; N-Nil														

*RNI- Reference Nutrient Intakes according to Department of Health, UK (2010) ⁽³⁰⁷⁾

^a -WHO recommendation for Sodium < 2 g Na/day or 5g salt/day for children ⁽³⁰⁹⁾

**Vitamin A: 400-600 µg/day ⁽³⁰⁷⁾

Table 23 shows that at six months for both groups micronutrient composition exceeded the daily RNI values. With respect to sodium, children's daily sodium intake increased from baseline and was above the WHO guidelines for children < 2 g Na⁺/day or one teaspoon (5 g) of table salt/day.

Table 24. Vitamin composition of main food groups for intervention and control group at six months

NUTRIENT ANALYSIS OF FOOD GROUPS (VITAMINS)												
FOOD ITEM	Six- Months											
	Vitamin D (µg)	Vitamin E (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Tryptophan (mg)	B6 (mg)	B12 (µg)	Folate (µg)	Pantothenate (mg)	Biotin (µg)	Vitamin C (mg)
Dairy Products												
Intervention 453.09	3.62	6.03	0.86	5.57	3.62	55.73	1.59	19.94	416.84	9.61	52.10	13.59
Control 517.24	4.14	6.88	0.98	6.36	4.14	63.61	1.81	22.71	475.82	10.96	59.48	15.52
Fruit												
Intervention: 270.25	0.00	0.93	0.28	0.13	1.49	0.62	0.51	0.00	72.08	0.81	7.03	157.56
Control: 259.08	0.00	0.89	0.27	0.13	1.42	0.59	0.49	0.00	69.09	0.78	6.74	151.04
Vegetables												
Intervention: 172.58	0.00	5.28	0.47	0.16	3.45	2.05	0.88	0.19	251.27	1.19	3.12	229.6
Control: 206.23	0.00	6.31	0.55	0.19	4.12	2.45	1.05	0.23	300.27	1.42	3.73	274.43
Legumes												
Intervention: 59.81	0.00	0.46	0.10	0.04	1.05	1.00	0.10	0.00	41.45	1.23	1.25	2.69
Control: 46.81	0.00	0.38	0.01	0.03	0.82	0.79	0.08	0.00	32.44	0.96	0.98	2.11
Starch												
Intervention: 94.18	0.79	2.23	0.58	0.53	9.42	3.29	0.84	1.22	187.65	0.61	5.65	64.04
Control: 75.78	0.64	1.79	0.47	0.42	7.58	2.65	0.75	0.98	150.99	0.49	4.55	51.53
Meat												
Intervention: 69.60	0.52	0.17	0.11	0.29	4.91	5.67	0.4	2.78	16	1.31	2.78	0.00
Control: 82.88	0.62	0.21	0.13	0.35	5.85	6.75	0.48	3.31	19.06	1.56	3.31	0.00
Seafood												
Intervention: 5.60	0.01	0.02	0.01	0.17	0.05	0.20	0.01	0.56	0.84	0.02	0.05	0.28
Control: 7.09	0.01	0.03	0.01	0.21	0.07	0.26	0.02	0.71	1.06	0.02	0.07	0.35
Fish												
Intervention: 61.23	4.61	1.13	0.16	0.22	6.88	5.06	0.53	6.43	15.18	0.89	5.66	Tr
Control: 23.55	1.77	0.43	0.06	0.08	2.64	1.95	0.2	2.47	5.84	0.34	2.17	Tr

Fats												
Intervention: 24.01	1.896	2.29	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Control: 4.88	1.96	2.37	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.00
Fast foods												
Intervention: 19.61	0.06	0.16	0.03	0.02	0.37	0.47	0.02	0.17	3.04	0.09	0.49	0.39
Control: 23.75	0.07	0.19	0.04	0.03	0.45	0.57	0.02	0.20	3.68	0.10	0.59	0.47
Sweets												
Intervention: 20.58	0.07	0.24	0.02	0.04	0.13	0.27	0.01	0.12	4.40	0.13	0.88	0.20
Control: 29.89	0.10	0.36	0.03	0.06	0.19	0.39	0.015	0.18	6.39	0.19	1.28	0.29
Savoury snacks												
Intervention: 28.93	0.00	2.09	0.09	0.07	0.92	0.38	0.27	0.00	8.68	0.27	N	12.44
Control: 39.26	0.00	2.85	0.12	0.10	1.26	0.51	0.36	0.00	11.78	0.36	N	16.88
Total intake:												
Intervention (units/day)	11.57	21.03	2.72	2.70	32.29	74.74	5.16	31.41	1017.43	16.15	79.02	480.79
Control (units/day)	9.31	22.69	2.67	2.66	28.54	80.52	5.27	30.79	1076.42	17.18	82.90	512.59
*RNI (4-14 years)	10µg	0.4mg/g PUFA	0.7-0.9	0.8-1.2	11-15	12mg/Kgbwt ^a	0.9-1.2	0.8-1.2	100-200	0.2 ^b	0.9 ^b	30-35

Key: Trace-Tr; N-Nil

*RNI- Reference Nutrient Intakes according to Department of Health, UK (2010) ⁽³⁰⁷⁾; a ⁽³¹⁰⁾ b ⁽³¹¹⁾

Table 24 illustrates that at six months children in both groups exceeded the daily recommended nutrient intake values for vitamins C, E, B and folate.

APPENDIX 3

Letters of acceptance from scientific journals for publication of manuscripts 2017-2020

List of publications in scientific journals

	Manuscript Title	Page
1.	Maria M Papamichael, Catherine Itsiopoulos, Nugroho H Susanto and Bircan Erbas, 2017. Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? A systematic review of observational studies. <i>Public Health Nutr.</i> 2017 Oct 20(15), 2722–2734. doi.org/10.1017/S1368980017001823. Epub 2017 Aug 14.	652
2.	Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas and Catherine Itsiopoulos, 2018. A Clinical Trial of Mediterranean Diet Enriched with Fatty Fish in Pediatric Asthma: Study Protocol. <i>J Pharmacy Pharmacol.</i> 6: 225-239 doi: 10.17265/2328-2150/2018.03.004.	653
3.	Maria Michelle Papamichael, Som Kumar Shrestha, Catherine Itsiopoulos, Bircan Erbas, 2018. The role of fish intake on asthma in children: A meta-analysis of observational studies. <i>Pediatr Allergy Immunol.</i> 2018; June 29:350–360. Epub 2018 March 5 doi: 10.1111/pai.12889.	654
4.	Papamichael M.M., Katsardis Ch., Lambert K., Tsoukalas D., Koutsilieris M., Erbas B., Itsiopoulos C., 2019. Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial. <i>J Hum Nutr Diet.</i> 2019 Apr; 32(2): 185-197. doi: 10.1111/jhn.12609. Epub 2018 Oct 30.	655
5.	Maria Michelle Papamichael, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, Dimitris Tsoukalas, 2019. Urinary organic acids as biomarkers in the assessment of pulmonary function in children with asthma. <i>Nutr Res.</i> 2019 Jan; 61: 31 – 40. doi:10.1016/j.nutres.2018.10.004. Epub 2018 Oct 13.	656
6.	Maria Michelle Papamichael; Charis Katsardis; Dimitris Tsoukalas; Bircan Erbas; Catherine Itsiopoulos, 2019. Weight status and respiratory health in asthmatic children. <i>Lung</i> 2019 Dec;197(6):777-782. doi: 10.1007/s00408-019-00273-w [Epub 2019 Sep 14]	657
7.	Maria M. Papamichael, Catherine Itsiopoulos, Katrina Lambert, Charis Katsardis, Dimitris Tsoukalas, Michael Koutsilieris and Bircan Erbas, 2019. The impact of vitamin D status on lung function in asthmatic children adhering to a Mediterranean diet enriched with fatty fish. <i>Nutr Res (Currently under review)</i>	658
8.	Papamichael MM, Theodoraki EM (2019). A comprehensive study of assessing a distribution's normality. Availability of statistical methods in nine software tools. <i>Statistical Papers (Springer).</i> (Currently under review)	659
9.	Thanasoula M, Sarandi E, Anamaterou C, Papakonstantinou E, Geraci F, Papamichael M.M., Itsiopoulos C, Tsoukalas D, Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases' in Makowski G. (ed) <i>Advances in Clinical Chemistry</i> (Elsevier). (Currently under review)	660

Letters of manuscript acceptance for publications presented in chronological order

1. Maria M Papamichael, Catherine Itsiopoulos, Nugroho H Susanto and Bircan Erbas, 2017. **Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? A systematic review of observational studies.** *Public Health Nutr.* 2017 Oct 20(15), 2722–2734. doi.org/10.1017/S1368980017001823. Epub 2017 Aug 14.

Public Health Nutrition

Decision Letter (PHN-REV-2017-0078.R1)

To: sassipap@hotmail.com

CC:

Subject: Public Health Nutrition- Decision on Manuscript ID PHN-REV-2017-0078.R1

Body: 19-Jun-2017

Dear Author,

Thank you for submitting your manuscript entitled "Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? : A systematic review of observational studies." to Public Health Nutrition. **I am pleased to confirm that your manuscript is acceptable for publication in Public Health Nutrition in its current form.**

If you have not yet done so, please complete and return the journal's grant of licence form as soon as possible. Once we have received this, your manuscript will enter the production process and the proofs will be sent to you in due course.

PLEASE NOTE: Public Health Nutrition offers authors the option to publish their article through an Open Access model (see <https://www.cambridge.org/core/services/open-access-policies/introduction-to-open-access>), upon payment of an Article Processing Charge (APC's). For the APC's for Public Health Nutrition, please visit the link at the bottom of this email.

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If you or your institution are planning to issue a press release for this article please inform the editorial office at phn.edoffice@cambridge.org. For more information on how you can promote your work via social media, please visit our social media guide here: <http://ow.ly/bhZY30avSus>

Thank you for submitting your interesting study to Public Health Nutrition.

Sincerely,

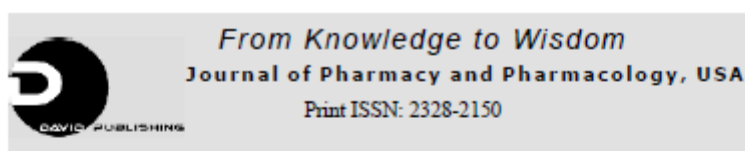
Dr. Carl Lachat

Deputy Editor, Public Health Nutrition

phn.edoffice@cambridge.org

Date Sent: 19-Jun-2017

2. Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas and Catherine Itsiopoulos, 2018. **A Clinical Trial of Mediterranean Diet Enriched with Fatty Fish in Pediatric Asthma: Study Protocol.** *J of Pharmacy Pharmacol.* 6: 225-239 doi: 10.17265/2328-2150/2018.03.004.



Paper Acceptance Notice

January 22, 2018

Dear Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas and Catherine Itsiopoulos,

We are pleased to inform you that your paper "A Clinical Trial of Mediterranean Diet Enriched with Fatty Fish in Pediatric Asthma: Study Protocol" has passed the examination of our journal's committee, and it will be published recently, with timely revision and payment.

Thank you for entrusting the publishing of your academic papers on *Journal of Pharmacy and Pharmacology* (USA). Should you have any question, please don't hesitate to contact us by e-mail.

Sincerely,
 Editorial Department
Journal of Pharmacy and Pharmacology (ISSN 2328-2150, USA)
 David Publishing Company, USA

Address of Headquarter: David Publishing Company, 616 Corporate Way, Suite 2-4876, Valley Cottage, NY 10989, USA
 Tel: 1-323-9847526; Fax: 1-323-9847374
 E-mail: pharmacy@davidpublishing.com Website: <http://www.davidpublisher.com>

3. Maria Michelle Papamichael, Som Kumar Shrestha, Catherine Itsiopoulos, Bircan Erbas, 2018. **The role of fish intake on asthma in children: A meta-analysis of observational studies.** *Pediatr Allergy Immunol.* 2018; June 29:350–360. Epub 2018 March 5. doi: 10.1111/pai.12889.

Pediatric Allergy and Immunology

Decision Letter (PAI-17-R-0259.R2)

From: paied@wiley.com

To: sassipap@hotmail.com, somkr.stha@gmail.com, C.itsiopoulos@latrobe.edu.au, b.erbas@latrobe.edu.au

Subject: Pediatric Allergy and Immunology - Manuscript PAI-17-R-0259.R2

Body:

Dear Mrs. Papamichael,

We are delighted to inform you that your manuscript, 'The role of fish intake on asthma in children: A meta-analysis of observational studies.' (PAI-17-R-0259.R2), has been accepted for publication in Pediatric Allergy and Immunology.

Your paper is now sent to the production office. You will receive proofs by e-mail in the near future. Please return your author corrections as quickly as possible.

Once your paper is sent to the production office, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

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Tel: +632-855 8714; +632 855 8790
Fax: +632 325 0768

As part of the Journal's continued commitment to its authors, the Editorial Office and Publisher wish to keep you informed about what will happen next and, as the attached paper contains important information regarding journal publication and services for authors, you may wish to save it for future reference.

Kindest regards

Dr Philippe Eigenmann
Editor-in-Chief
Pediatric Allergy and Immunology

Date Sent: 05-Mar-2018

4. Papamichael M.M., Katsardis Ch., Lambert K., Tsoukalas D., Koutsilieris M., Erbas B., Itsiopoulos C., 2019. **Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial.** *J Hum Nutr Diet.* 2019 Apr; 32(2): 185-197. doi: 10.1111/jhn.12609. Epub 2018 Oct 30.

In Production: Your article accepted in Journal of Human Nutrition and Dietetics

cs-author@wiley.com 10.10.18

Dear Maria Papamichael,

Article ID: JHN12609

Article DOI: 10.1111/jhn.12609

Internal Article ID: 16060914

Article: Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: A randomized controlled trial.

Journal: Journal of Human Nutrition and Dietetics

Congratulations on the acceptance of your article for publication in Journal of Human Nutrition and Dietetics.

Your article has been received and the production process is now underway. We look forward to working with you and publishing your article. Using Wiley Author Services, you can track your article's progress.

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Wiley Author Services

5. Maria Michelle Papamichael, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, Dimitris Tsoukalas, 2019. **Urinary organic acids as biomarkers in the assessment of pulmonary function in children with asthma.** *Nutr Res.* 2019 Jan; 61: 31 – 40. doi: 10.1016/j.nutres.2018.10.004. Epub 2018 Oct 13.

From: "Bruce Watkins (Nutrition Research)" <Evisesupport@elsevier.com>

Date: 6 October 2018 at 6:12:43 am AEST

To: C.Itsiopoulos@latrobe.edu.au

Subject: Your manuscript NR_2018_311_R3 has been accepted

Reply-To: baw@purdue.edu

Ref: NR_2018_311_R3

Title: Urinary organic acids as biomarkers in the assessment of pulmonary function in children with asthma.

Journal: Nutrition Research

Dear Dr. Itsiopoulos,

We are pleased to inform you that your paper **has been accepted for publication**. Now that your manuscript has been accepted for publication it will proceed to copy-editing and production.

In the near future you will receive page proofs. The proofs will consist of a PDF of your article, instructions, and a query page (if applicable). After you receive these proofs, please review them carefully and provide all necessary information to the publisher within 48 hours to prevent any delay in publication.

Thank you for submitting your work to Nutrition Research. We hope you consider us again for future submissions.

Kind regards,

Nutrition Research, Editorial Office

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Elsevier B.V., Radarweg 29, 1043 NX Amsterdam, The Netherlands, Reg. No. 33156677.

6. Maria Michelle Papamichael; Charis Katsardis; Dimitris Tsoukalas; Bircan Erbas; Catherine Itsiopoulos (2019). **Weight status and respiratory health in asthmatic children.** *Lung* (2019) 197:777-782 DOI 10.1007/s00408-019-00273-w

View Letter

Date: 03 Sep 2019
To: "Maria Michelle Papamichael" sassipap@hotmail.com
From: "Lung (LUNG)"
kanishkaa.sridhar@springernature.com
Subject: Decision on your manuscript #LUNG-D-19-00337R1

Dear Mrs Papamichael,

We are pleased to inform you that your manuscript, "Weight status and respiratory health in asthmatic children", **has been accepted for publication in Lung.**

Please remember to quote the manuscript number, LUNG-D-19-00337R1, whenever inquiring about your manuscript.

Sincerely,

Dr. F. Dennis McCool
Editor-in-Chief,
Lung
Professor of Medicine, Brown University

Reviewer #1: All the questions have been addressed

Currently under review

7. Maria M. Papamichael, Catherine Itsiopoulos, Katrina Lambert, Charis Katsardis, Dimitris Tsoukalas, Michael Koutsilieris and Bircan Erbas (2019). **The impact of vitamin D status on lung function in asthmatic children adhering to a Mediterranean diet enriched with fatty fish.** *Nutrition Research* (Manuscript ID: NR 2019 902).

Track your co-authored submission to Nutrition Research

Nutrition Research <Evisesupport@elsevier.com>
Thu 10/10/2019 1:53 PM

Dear Mrs Papamichael,

Submission no: NR_2019_902

Submission title: The impact of vitamin D status on lung function in asthmatic children adhering to a Mediterranean diet enriched with fatty fish.

Corresponding author: Dr Catherine Itsiopoulos

Listed co-author(s): Mrs Maria Papamichael, Ms Katrina Lambert, Dr Charis Katsardis, Dr. DIMITRIS TSOUKALAS, Dr Bircan Erbas

Dr Itsiopoulos has submitted a manuscript to Nutrition Research and listed you as a co-author. This email is to let you know we will be in contact with updates at each decision stage of the submission process.

The link below takes you to a webpage where you can sign in to our submission system using your existing Elsevier profile credentials or register to create a new profile. You will then have the opportunity to tailor these updates and view reviewer and editor comments once they become available.

http://www.evisesupport.com/profile/api/navigate/NR?resourceUrl=%2Fco-author%2F%3Fdgcid%3Dinvite_email_coauthoroutreach02715317%23%2FNR%2Fsubmission%2FNR_2019_902

If you are not a co-author of this manuscript, please contact Researcher Support at: <https://service.elsevier.com>

Thank you very much for your submission and we will be in touch as soon as we have any news to share.

Nutrition Research

Currently under review

8. Papamichael MM, Theodoraki EM (2019). **A comprehensive study of assessing a distribution's normality. Availability of statistical methods in nine software tools.** *Statistical Papers* (Springer Manuscript ID:STPA-D-19-00467).

STPA-D-19-00467: Submission Confirmation for A comprehensive study of assessing a distribution's normality. Availability of statistical methods in nine software tools.

Statistical Papers (STPA) <em@editorialmanager.com>

Mon 4/11/2019 10:04 PM

Dear Mrs Papamichael,

Your submission entitled "A comprehensive study of assessing a distribution's normality. Availability of statistical methods in nine software tools." has been received by Statistical Papers

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is <https://www.editorialmanager.com/stpa/>.

The submission id is: STPA-D-19-00467

Please refer to this number in any future correspondence.

Thank you for submitting your work to our journal.

Kind regards,

Editorial Office
Statistical Papers

Currently under review

9. Thanasoula M, Sarandi E, Anamaterou C, Papakonstantinou E, Geraci F, **Papamichael M.M**, Itsiopoulos C, Tsoukalas D, **Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases** in Makowski G. (ed) *Advances in Clinical Chemistry (Elsevier)*.

From: Makowski, Gregory <Gregory.Makowski@hhchealth.org>
Sent: Friday, January 3, 2020 10:13 PM
To: dsoukalas@einum.org
Cc: Bryant, Shellie (ELS-OXF) <s.bryant@elsevier.com>
Subject: ACC0941

Article Ref. No.: ACC0941
 Article Type: Invited Review
 Title: Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases.
 Author(s): Thanasoula, Sarandi, Anamaterou, Papakonstantinou, Geraci, Papamichael, Itsiopoulos, Tsoukalas

Dear Dr Tsoukalas

Receipt of the above manuscript to the Advances in Clinical Chemistry series is hereby acknowledged.

To expedite the editorial process, please submit the names and email addresses of 3-5 colleagues who could provide an unbiased review of the manuscript.

Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors have reviewed the manuscript and concur with the submission. Please be aware that your privileged communication will be distributed to reviewers and that return of any original material is not guaranteed.

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Dr. Gregory Makowski
 Editor, Advances in Clinical Chemistry

gregory.makowski@hhchealth.org

Statement of Co-Authorship

Publication 1: Type of Publication Book Chapter

Statement from the co-authors confirming the authorship contribution of the PhD Candidate.

To the Dean of La Trobe University Graduate Research School,

We hereby confirm that Mrs. Maria Michelle Papamichael is a co-author of the publication «Thanasoula M, Sarandi E, Anamaterou C, Papakonstantinou E, Geraci F, Papamichael M.M., Itsiopoulos C, Tsoukalas D, 'Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases' in Makowski G. (ed) *Advances in Clinical Chemistry* .Elsevier », which is under review.

We certify that Mrs. Papamichael made the following contributions:

- Application of search strategy and selection criteria
- Completion of quality assessment
- Analysis and interpretation of data;
- Assessed the level of evidence for outcomes of this review;
- Writing the manuscript and will contribute to response to reviewers' comments.

List of co-authors

Date

Author 1 Thanasoula Maria



5/2/2020

Author 2 Sarandi Evangelia



5/2/20.

Author 3 Anamaterou Chrisanthi



03/02/20

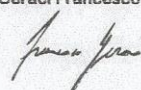


Author 4 Papakonstantinou Evangelos

10/01/20

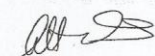
Author 5 Geraci Francesco

15/02/20



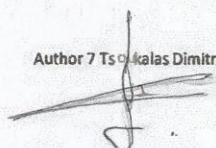
Author 6 Itsiopoulos Catherine

19/02/20



Author 7 Tsoukalas Dimitris

05/02/20



APPENDIX 4 CONFERENCE PARTICIPATION

Abstracts accepted and presentation at international conferences 2016-2020:

1. M. M. Papamichael and C. Itsiopoulos, 2016. “*The prophylactic potential of a Mediterranean dietary pattern enriched with fatty fish in improving respiratory function in asthmatic children: a randomized controlled trial*” presented as **poster** at 1st World Conference on the Mediterranean diet (IFMED) 6-8 July, 2016 Milano, Italy.
2. M. M. Papamichael, Ch. Katsardis, B. Erbas, C. Itsiopoulos, 2017. “*Does a Mediterranean dietary pattern enriched with fatty fish improve respiratory function and reduce asthma symptoms in children?: a randomized controlled trial*” presented as **e-poster** at 11th European Nutrition & Dietetics Conference, June 29- July 1 2017, Madrid, Spain. Abstract published in *J Food Nutr Disord* 2017, 6(3): 93.
3. Maria M. Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2017. “*Does a Mediterranean dietary pattern enriched with fatty fish improve respiratory function and reduce asthma symptoms in children? A Randomized Controlled Trial. Report of baseline results*” presented as **e-poster** at the 3rd International Conference on Respiratory and Pulmonary Medicine July 17-18, 2017, Melbourne, Australia
4. M.M. Papamichael, Ch. Katsardis, D. Tsoukalas, B.Erbas, C. Itsiopoulos, 2017. “*A Mediterranean diet enriched with ω 3-polyunsaturated fatty acids in the management of paediatric asthma. A Randomised Control Trial*” presented as **e-poster** at 14th International Conference on Clinical Nutrition, July 27-29 2017, Rome, Italy. Abstract published in *J Nutr Disorders Ther* 2017, 7(3): 88
5. Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2017.” *Can dietary Omega 3 fatty acids reduce asthma symptoms in children? Preliminary results of a Randomized Controlled Trial*” presented as **video** at 10th American Pediatric Healthcare & Pediatric Infectious Diseases Congress, September 20-22 Toronto, Canada. Abstract published in *Current Pediatric Research* in conference proceedings page 44.

6. M.M. Papamichael, Ch. Katsardis, D. Tsoukalas, B. Erbas, C. Itsiopoulos, 2017. “*Greek children suffering from asthma abandon Mediterranean dietary pattern: Baseline results*” presented as **video** at 15th world Congress on Advances in Nutrition, Food Science & Technology, September 11-12, 2017 Edinburgh, Scotland.

7. Papamichael Maria, Katsardis Charis , Tsoukalas Dimitrios , Erbas Bircan, Itsiopoulos Catherine, 2017. “*A randomized controlled trial on the impact of a Mediterranean diet enriched with fatty fish on asthma in Greek children: Study Protocol*” presented as **poster** at 10th European Federation of Dietitians (EFAD) September 29-30 2017 Rotterdam, Netherlands.

8. Maria Papamichael, Charis Katsardis, Dimitrios Tsoukalas, Bircan Erbas and Catherine Itsiopoulos, 2017. “*A Mediterranean diet enriched with omega 3-polyunsaturated fatty acids in the management of pediatric asthma: A randomized controlled trial*” presented as **e-poster** at 17th Global Dietitians and Nutritionists Annual Meeting October 02-03, 2017 Kuala Lumpur, Malaysia. Abstract published in *J Nutr Food Sci* 2017, 7:(6). doi: 10.4172/2155-9600-C1-051.

9. Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2018. “*A clinical trial on the efficacy of a Mediterranean Diet enriched with fatty fish in the management of paediatric asthma: Preliminary Results*” presented as an **oral presentation** at 14th Pan Hellenic Nutrition & Dietitians Conference, November 24-27 2017, Athens Greece. Abstract published in *Clinical Nutrition (ESPEN)*, April 2018: 184.

10. Papamichael Maria Michelle, Tsoukalas Dimitris, Erbas Bircan, Itsiopoulos Catherine, Koutsilieris Michael, Katsardis Charis, 2018. “*The effect of a Mediterranean diet enriched with fatty fish in paediatric asthma. A Randomized Controlled Trial*” presented as an **oral presentation** at 12th PanHellenic College of Pediatrics Conference Feb 2-4, 2018 Athens, Greece.

11. Papamichael Maria Michelle, Katsardis Charis, Koutsilieris Michael, Tsoukalas Dimitris, Lambert Katrina , Erbas Bircan , Itsiopoulos Catherine, 2018. “*The role of dietary $\Omega 3$ fatty acids in paediatric asthma*” presented as **poster** at 7th Annual Middle East Congress in Clinical Nutrition, May 11-13th, 2018 Athens, Greece.

12. Papamichael Maria Michelle, Katsardis Charis, Koutsilieris Michael, Tsoukalas Dimitris, Lambert Katrina, Erbas Bircan, Itsiopoulos Catherine, 2018. “*Fatty fish (Ω 3)- A diet therapy for paediatric asthma?*” presented as a **video** at the World Congress on Nutrition & Dietetics, June 18-19 2018, Paris France. Abstract published in *J Clin Nutr Dietetics* 2018; 4: 34. doi:10.4172/2472-1921-C1-002

13. Papamichael Maria Michelle, Katsardis Charis, Koutsilieris Michael, Tsoukalas Dimitris, Lambert Katrina, Erbas Bircan, Itsiopoulos Catherine, 2018. “*The prophylactic potential of fatty fish consumption on airway inflammation in childhood asthma*” presented as a **video** at 17th American Pediatric HealthCare & Infectious Diseases Congress June 27-28, 2018, Vancouver, Canada.

14. Papamichael Maria Michelle, Katsardis Charis, Koutsilieris Michael, Tsoukalas Dimitris, Lambert Katrina, Erbas Bircan, Itsiopoulos Catherine, 2018. “*Omega 3 fatty acids- A new therapeutic target for childhood asthma?*” presented as a **video** at 14th International Congress on Advances in Natural Medicines, Nutraceuticals & Neurocognition, July 19-20, 2018, London, UK.

15. Maria M. Papamichael, Katrina Lambert, Dimitris Tsoukalas, Michael Koutsilieris, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, 2019. “*The importance of vitamin D status on lung function in asthmatic children*” presented as a **video** at 3rd World Congress on Nutrition, Dietetics & Nutraceuticals, Feb 25-26, 2019, Prague, Czech. Abstract published in *J Clin Nutr Diet* 2019 5:38. doi: 10.4172/2472-1921-C1-005.

16. Maria M. Papamichael, Katrina Lambert, Dimitris Tsoukalas, Michael Koutsilieris, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, 2019. “*The synergistic effect of vitamin D in pediatric asthma*” presented as a **video** at 20th International Congress on Nutrition & Health, Mar 28-30, 2019, Stockholm, Sweden.

17. Dimitris Tsoukalas, Gerasimos Tsilimidos, **Maria Michelle Papamichael**, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, 2019. “*Precision medicine advances in childhood atopic diseases: Results from atopic dermatitis and asthma*” presented **orally** at the World Summit of Pediatrics, June 20-23, 2019, Berlin, Germany.

18. Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2019. "*Urinary metabolomic profile of Greek asthmatic school-children*" presented as an **e-poster** at 28th International Conference on Pediatrics Health, Aug 12-13, 2019 Rome, Italy.

19. Maria Michelle Papamichael, Charis Katsardis, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2019. "*Overweight/obesity increases ventilatory capacity and reduces FeNO in asthmatic children*" presented as **video** at 2nd World Congress on COPD, Asthma & Lung Health Oct 9-10, 2019 Madrid, Spain

20. Maria Michelle Papamichael, Charis Katsardis, Katrina Lambert, Dimitris Tsoukalas, Bircan Erbas, Catherine Itsiopoulos, 2019. "*Does a Mediterranean diet enriched with fatty fish impact lung function in asthmatic children?*" presented **orally** at 15th Hellenic Nutrition & Dietetics Conference, Dec 13-15, 2019, Athens, Greece.

21. Papamichael Maria Michelle, Tsoukalas Dimitris, Erbas Bircan, Itsiopoulos Catherine, Katsardis Charis. "*The influence of body weight on lung function in asthmatic children*" presented **orally** at the Annual Conference of the College of Pediatrics Feb 14-15th, 2020, Athens, Greece

Abstracts

1. 1st World Conference on the Mediterranean Diet 6-8 July, 2016 Milano, Italy

THE PROPHYLACTIC POTENTIAL OF A MEDITERRANEAN DIETARY PATTERN ENRICHED WITH FATTY FISH IN IMPROVING RESPIRATORY FUNCTION IN ASTHMATIC CHILDREN: A RANDOMIZED CONTROLLED TRIAL

M. M. Papamichael¹ Phd.cand; Assoc. Prof. C. Itsiopoulos¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

Abstract

Background and Aims: Asthma has rapidly become the most frequent chronic disease in children globally, placing significant disease burden as the most common reasons for hospitalisation, absence from school and work for sufferers and their parents/carers, respectively. Managed primarily symptomatically with medications as there is no known cure. Emerging evidence indicates that diet and lifestyle play a role in the aetiology and management, with potential for a protective effect of a Mediterranean diet. Dietary clinical trials are lacking. We aim to investigate whether fatty fish consumption as part of a Mediterranean dietary pattern improves pulmonary function in asthmatic children.

Methods: A parallel 6 month randomized controlled dietary intervention study will be conducted in asthmatic children, aged 5-12 years attending a paediatric respiratory clinic in Athens, Greece. The intervention will include two fatty fish meals (150g cooked) per week within the context of a Greek Mediterranean dietary pattern. The control group will consume their usual diet. Assessments at baseline and 6 month follow-up will include pulmonary function using spirometry (FEV₁), asthma symptoms using the Child Asthma Control test (CACT), quality of life using the Paediatric Asthma Quality of life (PAQOL), medication use, days hospitalized and absent from school. A food frequency questionnaire will be used to assess dietary intake and adherence to the Mediterranean dietary pattern using the KIDMED index.

Applications: This study will identify the potential of a Mediterranean diet supplemented with fatty fish in ameliorating asthma symptoms and improving pulmonary function in children with asthma.

Trial Registration: ANZCTR.org.au: ACTRN12616000492459p

Keywords: asthma, children, Mediterranean diet, lung function

Topic: 4. Promoting the Mediterranean Diet lifestyle pattern

Abstract published in *1st World Conference on the Mediterranean diet Conference Proceedings Journal* in poster sessions 4, 6-8 July, 2016 Milano, Italy



6-7-8 JULY 2016
Auditorium Testori,
Piazza Città di Lombardia
MILAN

1st World Conference on the
Mediterranean Diet

ABSTRACTS

Organized by
IFMeD International Foundation of Mediterranean Diet
V. Scudato and C. Scudato - Firenze

On the occasion of the
2016 International Year of Pulses

REVITALIZING THE MEDITERRANEAN DIET

From a healthy dietary pattern
to a healthy Mediterranean
sustainable lifestyle

with the technical support of
Ministero della Sanità

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patronage of
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SENC, FEDERATION OF AFRICAN NUTRITION SOCIETIES, FORUM MEDITERRANEO, ICAP, NUTRITION EDUCATION, FI, M, VIV, R. ILLUS, CIBER, CONEC

MEDITERRANEAN DIET IN ITALIAN RURAL AREAS: THE PAST AND THE PRESENT, F. Intorre; M.S. Foddai; E. Venneria; L. Barnaba; D. Ciarapica; L. Palomba; M. Zaccaria; E. Azzini; G. Maiani; A. Polito

Council for Agricultural Research and Economics - Research Centre for Food and Nutrition, Italy

Introduction The main features of the Mediterranean Diet have been initially identified in 1954 in the small rural area of Rofrano (National Park of Cilento, Vallo di Diano e Alburni), but they are now gradually being lost due to the spread of the western-type economy and technological society as well as the globalisation of food production and consumption, even in rural settings. The aim of this work is to compare the current food consumption of an Italian rural area with the dietary pattern of Rofrano described in the 1950s, as representative of the Italian rural areas.

Material & Methods This work shows the results obtained on 129 adult and elderly volunteers recruited in 3 different rural centres of the Majella National Park (Montenerodomo, Gamberale, Pizzoferrato). Food consumption was recorded by a validated food diary on 4 consecutive days, including 2 weekend days. Food intake was converted into nutrient intake using the Italian food composition tables. Anthropometric measurements were performed according to the standardized procedure (Lohman, 1988).

Results and Discussion The energy intake of Rofrano population was 2148 kcal, 20.6% of which provided by fats (Cresta et al, 1998). In our sample, the BMI of volunteers is 28.6 ± 5.0 kg/m², indicative of a mean condition of overweight; the total energy intake is 1802 ± 476 kcal and the percentage of energy provided by fats is 37.1%. The food consumption is different from that reported in Rofrano, where more than 40% of the active population was engaged in agriculture, which required considerable physical effort and adequate caloric intake guaranteed by cereals.

Conclusions Our preliminary results show a change in food consumption with regard to the energy deriving from products of vegetable and animal origin, maybe due to changes in food production system and globalization. The high prevalence of overweight and obesity confirms literature data on the increase of this phenomenon also in rural areas, maybe due to the modern lifestyle.

References Cresta M et al. Quaderni della nutrizione 1963; 23: 1-24.

Lohman TG, et al. Anthropometric standardization reference manual. USA: Human Kinetics Books, 1988

Sponsorship This study was supported by the Italian Ministry of Agricultural, Food and Forestry Policies, in the framework of the TERRAVITA project.

THE PROPHYLACTIC POTENTIAL OF A MEDITERRANEAN DIETARY PATTERN ENRICHED WITH FATTY FISH IN IMPROVING RESPIRATORY FUNCTION IN ASTHMATIC CHILDREN: A RANDOMIZED CONTROLLED TRIAL, M. M. Papamichael¹ Phd.cand; Assoc. Prof. C. Itsiopoulos¹ PhD, ILa Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

Background and Aims: Asthma has rapidly become the most frequent chronic disease in children globally, placing significant disease burden as the most common reasons for hospitalisation, absence from school and work for sufferers and their parents/carers, respectively. Managed primarily symptomatically with medications as there is no known cure. Emerging evidence indicates that diet and lifestyle play a role in the aetiology and management, with potential for a protective effect of a Mediterranean diet. Dietary clinical trials are lacking. We aim to investigate whether fatty fish consumption as part of a Mediterranean dietary pattern improves pulmonary function in asthmatic children.

Methods: A parallel 6 month randomized controlled dietary intervention study will be conducted in asthmatic children, aged 5-12 years attending a paediatric respiratory clinic in Athens, Greece. The intervention will include two fatty fish meals (150g cooked) per week within the context of a Greek Mediterranean dietary pattern. The control group will consume their usual diet. Assessments at baseline and 6 month follow-up will include pulmonary function using spirometry (FEV1), asthma symptoms using the Child Asthma Control test (CACT), quality of life using the Paediatric Asthma Quality of life (PAQOL), medication use, days hospitalized and absent from school. A food frequency questionnaire will be used to assess dietary intake and adherence to the Mediterranean dietary pattern using the KIDMED index.

Applications: This study will identify the potential of a Mediterranean diet supplemented with fatty fish in ameliorating asthma symptoms and improving pulmonary function in children with asthma.

Trial Registration: ANZCTR.org.au: ACTRN12616000492459p

PLASMATIC ANTIOXIDANT STATE AND THE CONSUMPTION OF ANTIOXIDANT FOOD IN ELDERLY ADULTS FROM CATAMARCA –ARGENTINA, □ O.T.Barrionuevo¹; M.A Cornatosky¹; A.M.Barrionuevo¹, ¹ Facultad de Ciencias de la Salud Universidad Nacional de Catamarca, Argentina

Introduction: Eating habits are part of the culture of a society; they show deep roots that link man with his land and his customs and traditions. These eating habits are expressed in terms of consumption patterns and are acquired at childhood and remain present in adulthood. The purpose of this work was to calculate the association between Total Antioxidant State (TAS) in plasma of a group of Elderly Adults (EA) from Catamarca –Argentina and Eating Profile (EP), expressed as the adherence to habitual consumption of food rich in natural antioxidants characteristic of this part of the country. **Materials and Methods:** It is an epidemiological cross-section and descriptive study carried out in the Western Region (WR) of Catamarca. 66 EA (74.3±2.8 years old) from both sexes took part in the study. The anthropometric and biochemical parameters are compatible with high risk of Cardiovascular Disease (CD). The TAS value was determined by the colorimetric method used by ABTS®. To describe the antioxidant EP a questionnaire was designed to show the frequency of reliable consumption of a closed list of items of food rich in natural antioxidants and characteristic of the WR. Percentages of consumption (from 2 to 3 times a week) were calculated and a cluster analysis was carried out. The data obtained were analyzed with the SPSS 18 software. **Results and Discussion:** The TAS value was 1.45±0.13 mmol/L. Besides, 12.1% of the sample showed values of <1.31 mmol/L. The frequencies of consumption were: legumes (33%), walnuts (32%), raisins (26%) and olive oil (12%). They formed a low consumption cluster and their association with TAS was lineal, direct and weak (Eta=0.019). As regards olive oil, it is worth wondering the reasons behind its low consumption and to what extent to the population is aware of the guidance and recommendations about its intake. **Conclusions:** The work gives epidemiological tools for the design of appropriate actions to encourage the combined consumption of these products. It could also, together with further studies, help to validate a healthy diet.

2. 11th European Nutrition & Dietetics Conference Jun 29-Jul 1, 2017, Madrid, Spain

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M M Papamichael et al., J Food Nutr Disord 2017, 6:3(Suppl)
DOI: 10.4172/2324-9323-C1-003

11TH EUROPEAN NUTRITION AND DIETETICS CONFERENCE

June 29-July 01, 2017 Madrid, Spain

Does a Mediterranean dietary pattern enriched with fatty fish improve respiratory function and reduce asthma symptoms in children? A randomized controlled trial

M M Papamichael¹, Ch Katsardis², B Erbas² and C Itsiopoulos¹

¹La Trobe University, Australia

²National & Kapodistrian University of Athens, Greece

Background & Aim: Globally, asthma has rapidly become the most frequent allergic disease in children. It causes significant burden and is the most common reason for hospitalisation, absence from school and work for sufferers and their parents/carers, respectively. There is no cure for asthma; it can only be controlled by medication. Emerging evidence from observational studies indicate that diet and lifestyle play a role in the aetiology and management, with potential for a protective effect of a Mediterranean diet. However, randomized controlled trials are lacking. We aim to investigate whether fatty fish consumption as part of a Mediterranean dietary pattern improves pulmonary function and reduces asthma symptoms in children.

Method: A parallel Randomized Controlled Trial of 6 months duration is being conducted in asthmatic children, aged 5-12 years attending a paediatric respiratory clinic in Athens, Greece. The intervention includes two fatty fish meals (150 g cooked) per week as part of the Greek Mediterranean diet. The control group will consume their usual diet. Assessments at baseline and 6 month follow-up include pulmonary function using spirometry (FEV₁) and exhaled nitric oxide, asthma symptoms using the Asthma Control Questionnaire (ACQ), quality of life by the Paediatric Asthma Quality of life Questionnaire (PAQLQ), medication use and days hospitalized. A Food Frequency Questionnaire will be used to assess dietary intake and adherence to the Mediterranean dietary pattern will be assessed using the KIDMED index.

Applications: This study is important in establishing the effect of a Mediterranean diet enriched with fatty fish in the management of asthma in children.

Published in J Food Nutr Disord, 2017 6(3). DOI: 10.4172.232409323-C1-003.

3. 3rd International Conference on Respiratory & Pulmonary Medicine July 17-18, 2017 Melbourne, Australia

Title: DOES A MEDITERRANEAN DIETARY PATTERN ENRICHED WITH FATTY FISH IMPROVE RESPIRATORY FUNCTION AND REDUCE ASTHMA SYMPTOMS IN CHILDREN? A RANDOMIZED CONTROLLED TRIAL. REPORT OF BASELINE RESULTS.

Maria M. Papamichael¹; Charis Katsardis³; Dimitris Tsoukalas⁴; Bircan Erbas²; Catherine Itsiopoulos¹

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

² La Trobe University, Department of Public Health, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy.

Statement of the Problem: Emerging evidence from observational studies indicates that diet and lifestyle play a role in the aetiology and management of chronic diseases such as obesity and asthma in children, with a potential for protective effect of a Mediterranean diet. Previous studies have reported an association between overweight, development and severity of asthma in children.

Purpose: This is the first Randomized Controlled Trial (RCT) to investigate whether fatty fish consumption as part of a Mediterranean dietary pattern improves pulmonary function and reduces asthma symptoms in children. **Methodology:** Children aged 5-12 years, suffering with doctor-diagnosed 'mild asthma' were recruited from a paediatric asthma clinic in Athens, Greece and randomized equally into two groups. The intervention group is instructed to consume 2 fatty fish meals per week (at least 150g cooked fish/meal) over a period of 6 months. And the control group, their usual diet. **Findings:** Data analysis of baseline measurements reveals that in a sample of 72 Greek children, 54.2% are male and 45.8% female (mean age 8 ± 2 y.o). Anthropometric evaluation shows that 64% of children are 'normal' height and 36% 'tall'. Regarding bodyweight, 1% of children are 'severely underweight', 3% 'slightly underweight', 57% 'normal' weight, 28% 'overweight' and 11% 'obese' according to the Hellenic paediatric growth charts. **Conclusion & Significance:** This observation is important since BMI seems to be a major risk factor in paediatric asthma. Future public health strategies should focus on promoting a healthy diet similar to the Mediterranean diet, daily

physical activity and maintenance of a healthy weight in the management of childhood asthma.

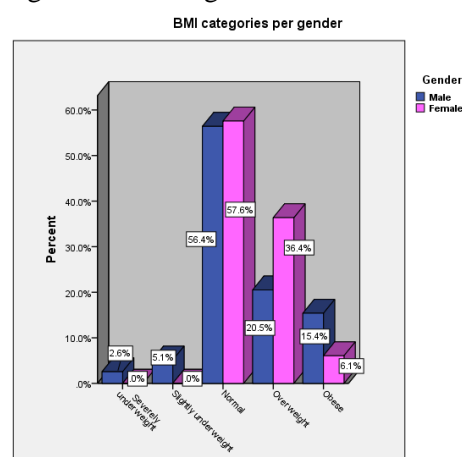


Figure 1. BMI distribution per gender at baseline

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3. Calder. P, 2015. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta*; 1851: 469–484.
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5. Chen et al, 2017. Effects of Childhood Asthma on the Development of Obesity among School aged Children. *Am J Respir Crit Care Med.* 2017. doi:10.1164/rccm.201608-1691

4. 14th International Conference on Clinical Nutrition, Jul 27-29, 2017, Rome, Italy

conferenceseries.com JOINT EVENT

M M Papamichael et al., J Nutr Disorders Ther 2017, 7:3(Suppl)
DOI: 10.4172/2161-0509-C1-007

13th International Congress on
Advances in Natural Medicines Nutraceuticals & Neurocognition
&
14th International Conference on Clinical Nutrition
July 27-29, 2017 Rome, Italy

A mediterranean diet enriched with ω 3-polyunsaturated fatty acids in the management of paediatric asthma: a randomised control trial

M M Papamichael¹, Ch Katsardis², D Tsoukalas³, B Erbas¹ and C Iliopoulos¹

¹La Trobe University, Australia

²National & Kapodistrian University of Athens, Greece

³European Institute of Nutritional Medicine, Italy

Asthma is an inflammatory disease in the lungs which, over the past thirty years has escalated in children. Considerable interest exists in the therapeutic potential of dietary omega 3 fatty acids, due to anti-inflammatory and immune-modulating effects on asthma. However, studies performed till date are inconclusive and this requires further exploration. This six month randomized controlled trial aims to investigate whether fatty fish, as part of the Greek Mediterranean diet reduces asthma symptoms in children. A sample of 64 children was recruited from a paediatric asthma clinic in Athens, Greece. Participant children will be randomized into two groups. The intervention group is required to consume two meals of fatty fish (≥ 150 gr cooked fish) per week over a period of 6 months in the context of the Greek Mediterranean diet. The control group will consume their usual diet. Outcome measures will be assessed at base-line and at the end of six months. Questionnaires will be used to collect socio-demographics data, medical information, dietary habits, asthma control and quality of life details. Pulmonary function will be assessed using spirometry and exhaled nitric oxide. In addition, blood and urine tests will be examined to assess patient's metabolic profile, antioxidant status, plasma fatty acid composition and Vitamin D. This study intends to establish whether fatty fish consumption can be used as an adjunct therapy in the management of asthma in children.

Published in J Nutr Disorders Ther 2017, 7 (3). Doi: 10.4172/2161-0509-C1-007.

5. **10th American Pediatric Healthcare & Pediatric Infectious Diseases Congress, September 20-22 Toronto, 2017 Canada.**

10TH AMERICAN PEDIATRICS HEALTHCARE & PEDIATRIC INFECTIOUS DISEASES CONGRESS

September 20-22, 2017 | Toronto, Canada

Can dietary Omega 3 fatty acids reduce asthma symptoms in children? Preliminary results of a randomized controlled trial

Maria Michelle Papamichael¹, Charis Katsardis², Dimitris Tsoukalas³, Bircan Erbas¹ and Catherine Itsiopoulos¹

¹LaTrobe University, Australia

²National & Kapodistrian University of Athens, Greece

³European Institute of Nutritional Medicine, Italy

There is a general consensus that the global allergy epidemic in children is attributed to a changing environment including lifestyle and diet that is high in omega 6 fatty acids and low in omega 3 fatty acids. It has been postulated that omega 3 fatty acids may modulate the development of IgE mediated allergic disease and regulate immune responses. This is the first clinical trial to investigate fatty fish (in the context of the Greek Mediterranean diet) as an adjunct therapy for paediatric asthma. Children aged 5-12 years with doctor-diagnosed 'mild asthma' were recruited from a paediatric asthma clinic in Athens, Greece and randomized into two groups. The intervention group is instructed to consume two serves of fatty fish per week (at least 150 g cooked fish/serve) for six months and the control group, their usual diet. Questionnaires were used to collect information on medical, dietary, socio-demographic, asthma control and quality of life. Spirometry (FEV1) and exhaled nitric oxide (eNO) analysis were used to evaluate pulmonary function. Adherence to the Mediterranean dietary pattern

was assessed using the KIDMED score. Seventy-two children (54.2% boys, 45.8% girls) were successfully recruited. At baseline, 56.94% are 'normal' weight, 27.78% 'overweight' and 11.11% 'obese' according to the Hellenic Paediatric Growth Charts. In conclusion, children suffering with asthma might be at higher risk of becoming overweight and this in turn may affect asthma symptoms. Clinicians should recommend the importance of healthy eating in the prevention and management of overweight issues in paediatric asthma.

Published in Current Pediatric Research

<http://www.alliedacademies.org/conference-abstracts/scientific-tracks-abstracts/pediatric-healthcare-pediatric-infections-2017-proceedings.html>

**6. 15th World Congress on Advances in Nutrition, Food Science & Technology,
September 11-12, 2017 Edinburgh, Scotland**

**Title: Greek children suffering from asthma abandon Mediterranean dietary pattern:
Baseline results**

M.M. Papamichael¹; Ch. Katsardis³; D. Tsoukalas⁴; B.Erbas²; C. Itsiopoulos¹

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

² La Trobe University, Department of Public Health, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴European Institute of Nutritional Medicine, Rome, Italy

ABSTRACT

Statement of problem: The rapid rise in paediatric asthma has become a major public health concern. Apart from a genetic predisposition, poor dietary habits have been implicated as one of the environmental factors responsible for the asthma epidemic. Emerging evidence from observational studies has documented a reduction in asthma prevalence and wheezing in children consuming a Mediterranean diet. However, intervention trials investigating the association between food groups and dietary patterns in children are lacking.

The purpose of this RCT study is to investigate whether an increase in fatty fish consumption in the context of a Mediterranean diet reduces asthma symptoms in Greek children.

Methodology: Children aged 5-12 years with doctor-diagnosed ‘mild asthma’ were recruited from a paediatric asthma clinic in Athens, Greece and randomized into two groups. The intervention group is instructed to consume 2 serves of fatty fish per week (at least 150g cooked fish/serve) for 6 months. And the control group, their usual diet. Questionnaires are used to collect information on medical, dietary, socio-demographic, asthma control and quality of life. Respiratory function is evaluated using spirometry and exhaled nitric oxide analysis. KIDMED test is used to evaluate adherence to the Mediterranean dietary pattern.

Findings: At baseline, from a sample of 72 children (54.2% boys, 45.8% girls), mean KIDMED score is 5.38 ± 2.02 ; 21.1% of children have “Very low adherence”, 60.6% “Need for improvement” and 18.3% “Optimal Mediterranean diet” adherence according to the KIDMED test.

Conclusion & Significance: There is a clear trend of abandonment of the Mediterranean lifestyle in Greek children. Given the sustainability and overall health benefits of the Mediterranean dietary pattern, it is essential that public health strategies focus on its promotion. Future clinical trials are recommended to provide concrete evidence on the efficacy of the Mediterranean diet in the management of childhood asthma.

7. 10th European Federation of Dietitians (EFAD) September 29-30 2017 Rotterdam, Netherlands

Title: A randomized controlled trial on the impact of a Mediterranean diet enriched with fatty fish on asthma in Greek children: Study Protocol.

Papamichael Maria¹; Katsardis Charis³; Tsoukalas Dimitrios⁴; Erbas Bircan²; Itsiopoulos Catherine¹.

1) Department of Rehabilitation, Nutrition & Sport, La Trobe University, Melbourne, Australia

2) Department of Public Health, La Trobe University, Melbourne, Australia

3) Department of Medicine, National & Kapodistrian University of Athens, Athens, Greece

4) European Institute of Nutritional Medicine, Rome, Italy

Introduction: Emerging evidence indicates a potential prophylactic effect of a Mediterranean diet in childhood asthma.

Objective: To investigate whether fatty fish consumption reduces asthma symptoms in children.

Methodology: Greek Children aged 5-12 y.o with 'mild' asthma were recruited. The intervention group consumes ≥ 150 g fatty fish/meal, twice weekly for 6 months and the control, their usual diet.

Results

At baseline 39% of children are 'overweight/ 'obese' and 60.6% 'need for improvement/or medium adherence' to the Mediterranean diet according to the KIDMED test.

Conclusion: Future public health strategies should focus on promoting healthy eating, physical activity in the prevention of overweight/obesity and management of childhood asthma.

Key points

- A Mediterranean diet enriched with fatty fish might reduce asthma symptoms in children with pre-existing asthma
- The findings suggest that children suffering with 'mild asthma' may be at higher risk of overweight

7. 17th Global Dieticians & Nutritionists Annual Meeting October 2-3, 2017,
Kuala Lumpur, Malaysia

conferenceseries.com

Maria Papamichael et al., J Nutr Food Sci 2017, 7:6 (Suppl)
DOI: 10.4172/2155-9600-C1-051

17th GLOBAL DIETICIANS AND NUTRITIONISTS ANNUAL MEETING

October 02-03, 2017 Kuala Lumpur, Malaysia

A Mediterranean diet enriched with omega 3-polyunsaturated fatty acids in the management of pediatric asthma: A randomized controlled trial

Maria Papamichael¹, Ch Katsardis², D Tseoukalas², B Erbas³ and C Itsiopoulos¹

¹La Trobe University, Australia

²National & Kapodistrian University of Athens, Greece

³European Institute of Nutritional Medicine, Italy

The change in dietary patterns has contributed to the rise in obesity and asthma in children. Both chronic diseases are associated with co-morbidities, considerable disability, poor quality of life and increase in medical costs. Research studies have demonstrated that an elevated BMI is related to an increase in asthma risk and development of future exacerbations, less asthma control and an increase need for medication use. The purpose of this Randomized Controlled Trial is to investigate the effect of a Mediterranean diet enriched with fatty fish on asthma in Greek children. This is the first announcement of baseline results for this intervention study. Sample consists of 72 children aged 5-12 years with doctor-diagnosed mild-asthma; of which 54.2% are male and 45.8% females were recruited in this study. Children were randomized equally into two groups. The intervention group is instructed to consume 2 fatty fish meals (at least 150 g cooked fish/meal) per week over a period of 6 months. And the control group, their usual diet. Statistical analysis of baseline data reveals that 64% of children are normal height and 36% are tall. Regarding bodyweight, 1% of children are severely underweight, 3% are slightly underweight, 57% are normal weight, 28% are overweight and 11% are obese, according to Hellenic pediatric growth charts. This finding is significant since BMI seems to play a major role on asthma outcome in children. The effect of weight reduction in overweight asthmatic children might be of great value for current treatment guidelines and in alleviation of asthma symptoms.

9. 14th Pan Hellenic Nutrition & Dieticians Conference, November 24-27 2017, Athens Greece

Title: A clinical trial on the efficacy of a Mediterranean diet enriched with fatty fish in the management of paediatric asthma: Preliminary results

Maria Papamichael¹, Charis Katsardis³, Dimitrios Tsoukalas⁴, Bircan Erbas², Catherine Itsiopoulos¹

¹ La Trobe University, School of Allied Health, Melbourne, Australia.

¹ La Trobe University, School of Public Health, Melbourne, Australia.

³ National and Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy



ABSTRACT

Background & aims: Over the past 30 years, there has been a parallel increase in asthma and obesity in children which has become a major public health concern. Accumulating evidence from observational studies has documented that adherence to a Mediterranean diet is inversely related to asthma prevalence, wheezing and overweight/obesity in children. However, intervention trials investigating the association between food groups and dietary patterns in children are lacking and this requires further exploration. This is the first clinical trial to investigate the usefulness of fatty fish (in the context of the Greek Mediterranean diet) as an adjunct therapy for pediatric asthma.

Methods: Children aged 5–12 years with doctor-diagnosed ‘mild asthma’ were recruited from a pediatric asthma clinic in Athens, Greece and randomized into two groups. The intervention group is instructed to consume 2 serves of fatty fish per week (at least 150g cooked fish/serve) for 6 months and the control group, their usual diet. Questionnaires are used to collect information on medical, dietary, socio-demographic, asthma control and quality of life. Spirometry (FEV1) and exhaled nitric oxide analysis are used to evaluate pulmonary function. Adherence to the Mediterranean dietary pattern is assessed using the KIDMED test.

Results: At baseline, 72 children (54.2% boys, 45.8% girls), of which 56.9% are ‘normal’ weight, 27.8% ‘overweight’, 11.1% ‘obese’ according to the Hellenic Paediatric Growth Charts. Regarding adherence to the Mediterranean diet, mean KIDMED score is 5.38 ± 2.02 , 21.1% of children have ‘very low adherence’, 60.6% ‘need for improvement’ and 18.3% ‘optimal Mediterranean diet’ adherence.

Conclusions: Children suffering from asthma have low adherence to the Mediterranean diet and might be at higher risk of becoming overweight. Public health strategies should focus on promoting healthy eating in the prevention and management of overweight and asthma in children. Future clinical trials are recommended to replicate the findings.

Disclosure of interest: None declared.

9. 14th Pan Hellenic Nutrition & Dieticians Conference, November 24-27 2017, Athens Greece (Oral presentation in Greek)

ΤΙΤΛΟΣ: ΚΛΙΝΙΚΗ ΔΟΚΙΜΗ ΣΧΕΤΙΚΑ ΜΕ ΤΗΝ ΕΠΙΔΡΑΣΗ ΤΗΣ ΜΕΣΟΓΕΙΑΚΗΣ ΔΙΑΤΡΟΦΗΣ ΕΜΠΛΟΥΤΙΣΜΕΝΗ ΜΕ ΛΙΠΑΡΟ ΨΑΡΙ ΣΤΗ ΔΙΑΧΕΙΡΙΣΗ ΤΟΥ ΠΑΙΔΙΚΟΥ ΑΣΘΜΑΤΟΣ: ΠΡΟΚΑΤΑΡΚΤΙΚΑ ΑΠΟΤΕΛΕΣΜΑΤΑ.

Μαρία Παπαμιχαήλ¹; Χάρης Κατσαρδής³; Δημήτριος Τσουκαλάς⁴; Bircan Erbas²; Κατερίνα Ιτσιόπουλος¹

¹ Πανεπιστήμιο La Trobe, Σχολή Επιστήμων Υγείας, Μελβούρνης, Αυστραλία.

² Πανεπιστήμιο La Trobe, Σχολή Δημόσιας Υγείας, Μελβούρνης, Αυστραλία.

³ Εθνικό & Καποδιστριακό Πανεπιστήμιο Αθήνας, Ελλάδα

⁴ Ευρωπαϊκού Ινστιτούτο Διατροφικής Ιατρικής, Ρώμη, Ιταλίας

Εισαγωγή

Τα τελευταία 30 χρόνια παρατηρείται παράλληλη αύξηση του άσθματος και της παχυσαρκίας στα παιδιά, η οποία έχει καταστεί σημαντικό πρόβλημα στη δημόσια υγεία. Μελέτες παρατήρησης κατέδειξαν ότι η προσκόλληση στη Μεσογειακή διατροφή συσχετίζεται με την μείωση του επιπολασμού του άσθματος και του υπερβολικού βάρους. Εντούτοις, δοκιμές παρέμβασης που διερευνούν τη συσχέτιση μεταξύ διατροφικών προτύπων στα παιδιά είναι περιορισμένες και αυτό απαιτεί περαιτέρω διερεύνηση.

Σκοπός: να εξεταστεί ή κατανάλωση λιπαρών ψαριών (στο πλαίσιο της ελληνικής Μεσογειακής διατροφής) ως συμπληρωματική θεραπεία στο παιδικό άσθμα.

Μέθοδοι / Υλικά: Παιδιά ηλικίας 5-12 ετών με "ήπιο άσθμα" προσλήφθηκαν από μια κλινική παιδιατρικού άσθματος στην Αθήνα, και τυχαιοποιήθηκαν σε δύο ομάδες. Η ομάδα παρέμβασης καταναλώνει 2 μερίδες λιπαρών ψαριών εβδομαδιαίως (τουλάχιστον 150gr μαγειρεμένου ψαριού ανα γεύμα) για 6 μήνες και η ομάδα ελέγχου τη συνήθη διατροφή της. Ερωτηματολόγια χρησιμοποιούνται για τη συλλογή πληροφοριών σχετικά με τον ιατρικό, διατροφικό, κοινωνικοδημογραφικό, έλεγχο του άσθματος και την ποιότητα ζωής των παιδιών. Η σπιρομέτρηση (FEV1) και η ανάλυση εκπνεόμενου νιτρικού οξειδίου (eNO) χρησιμοποιούνται για την αξιολόγηση της πνευμονικής λειτουργίας. Η προσκόλληση στο Μεσογειακό πρότυπο διατροφής αξιολογείται μέσω του KIDMED σκορ.

Αποτελέσματα: Κατά την έναρξη, 72 παιδιά (54,2% αγόρια, 45,8% κορίτσια), εκ των οποίων 56,9% είναι «φυσιολογικού» βάρους, 27,8% «υπέρβαρα», 11,1% «παχύσαρκα» σύμφωνα με τις Ελληνικές Παιδιατρικές Καμπύλες Ανάπτυξης. Όσον αφορά την τήρηση της μεσογειακής διατροφής, το KIDMED σκορ είναι 5.38 ± 2.02 , το 21.1% των παιδιών έχουν «πολύ χαμηλής προσκόλλησης», 60.6% «χρήζει βελτίωσης» και το 18.3% «βέλτιστης προσκόλλησης».

Συμπεράσματα: Τα παιδιά που πάσχουν από άσθμα έχουν χαμηλή προσκόλληση στη μεσογειακή διατροφή και ενδέχεται να διατρέχουν μεγαλύτερο κίνδυνο να γίνουν υπέρβαρα. Οι στρατηγικές για τη δημόσια υγεία πρέπει να επικεντρωθούν στην προώθηση της υγιεινής διατροφής, στην πρόληψη και τη διαχείριση του υπερβολικού βάρους και του άσθματος στα παιδιά. Στις μελλοντικές κλινικές δοκιμές συνιστάται η επανάληψη των ευρημάτων.

Θέμα: Παιδική Διατροφή

Παρουσίαση: Προφορική.

10. 12th PanHellenic College of Pediatrics Conference, Feb 2-4, 2018, Athens, Greece

Title: The effect of a Mediterranean diet enriched with fatty fish in paediatric asthma. A Randomized Controlled Trial.

Papamichael Maria Michelle¹; Tsoukalas Dimitris⁴; Erbas Bircan²; Itsiopoulos Catherine¹; Koutsilieris Michael³; Katsardis Charis³

¹ La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

² La Trobe University, Department of Public Health, Melbourne, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy

Abstract

The aim of this study is to investigate the effect of fatty fish ($\Omega 3$) as an adjunct therapy in childhood asthma. A randomized controlled trial of six months duration was performed in 72 children (54.2% boys; 45.8% girls), 5-12 years old with 'mild asthma' from a clinic in Athens that were randomized to two groups. The intervention group consumed two fatty fish meals per week and the control group, their usual diet. Pulmonary function was assessed using spirometry and bronchial inflammation with exhaled Nitric Oxide analysis (eNO). Parents completed questionnaires to collect information regarding socio-demographics, medical history, asthma control, quality of life and dietary habits. Adherence to the Mediterranean diet was assessed using the KIDMED score. A statistically significant change in eNO was observed in the intervention group (95%CI: -27.39, -0.91; $\beta\eta\tau\alpha$ = -14.15; $p=0.037$) after adjusting for confounders such as age, sex, regular physical activity and BMI. One unit increase in fatty fish intake reduced bronchial inflammation by 14 ppb for children in the intervention group. No significant differences were observed for spirometry.

These findings suggest an inverse relationship between fatty fish intake and bronchial inflammation in asthmatic children.

10. 12th PanHellenic College of Pediatrics Conference, Feb 2-4, 2018, Athens, Greece
(Oral presentation in Greek)

Τίτλος: Η ΕΠΙΔΡΑΣΗ ΤΗΣ ΜΕΣΟΓΕΙΑΚΗΣ ΔΙΑΤΡΟΦΗΣ ΕΜΠΛΟΥΤΙΣΜΕΝΗ ΜΕ ΛΙΠΑΡΟ ΨΑΡΙ ΣΤΟ ΠΑΙΔΙΚΟ ΑΣΘΜΑ. ΜΙΑ ΤΥΧΑΙΟΠΟΙΗΜΕΝΗ ΚΛΙΝΙΚΗ ΜΕΛΕΤΗ.

Παπαμιχαήλ Μαρία Μισέλ¹; Τσουκαλάς Δημήτρης⁴; Erbas Bircan²; Ιτσιόπουλος Κατερίνα¹; Κουτσλιέρης Μιχαήλ³; Κατσαρδής Χαράλαμπος

¹ La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

² La Trobe University, Department of Public Health, Melbourne, Australia³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy

Περίληψη

Ο σκοπός της μελέτης είναι να εξετάσει την επίδραση των λιπαρών ψαριών (Ω3) ως συμπληρωματική θεραπεία στο παιδικό άσθμα. Μια τυχαιοποιημένη κλινική μελέτη των έξι μηνών πραγματοποιήθηκε σε 72 παιδιά (54,2% αγόρια), ηλικίας 5-12 ετών με «ήπιο άσθμα» από ιατρείο της Αθήνας, και τυχαιοποιήθηκαν σε δύο ομάδες. Η ομάδα παρέμβασης κατανάλωνε δυο μερίδες λιπαρών ψαριών εβδομαδιαίως και η ομάδα ελέγχου τη συνήθη διατροφή της. Η πνευμονική λειτουργία αξιολογήθηκε με τη σπιρομέτρηση και η βρογχική φλεγμονή με τη μέτρηση του εκπνεόμενου μονοξειδίου του αζώτου (eNO). Γονείς συμπλήρωσαν ερωτηματολόγια για τη συλλογή πληροφοριών σχετικά με τα δημογραφικά, το ιατρικό ιστορικό, τον έλεγχο του άσθματος, την ποιότητα ζωής και τις διατροφικές συνήθειες. Η προσκόλληση στο Μεσογειακό πρότυπο διατροφής αξιολογήθηκε μέσω του KIDMED σκορ. Στατιστικά σημαντική μεταβολή στη μέση τιμή του eNO παρατηρήθηκε για την ομάδα παρέμβασης ($p=0.037$; 95% CI: -27.39, -0.91; βήτα= -14,15) μετά την προσαρμογή για επιπρόσθετους παράγοντες που επηρεάζουν (ηλικία, φύλο, φυσική δραστηριότητα και ΔΜΣ). Μία μονάδα αύξηση στην πρόσληψη λιπαρών ψαριών μείωσε τη βρογχική φλεγμονή κατά 14 ppb στα παιδιά της ομάδας παρέμβασης. Δεν παρατηρήθηκε στατιστικά σημαντική διαφορά για τις μεταβλητές σπιρομέτρησης.

Με βάση τα παραπάνω ευρήματα φαίνεται ότι πιθανώς υπάρχει μια αντίστροφη σχέση μεταξύ πρόσληψης λιπαρών ψαριών και βρογχικής φλεγμονής στα ασθματικά παιδιά.

Προφορική ανακοίνωση.

11. 7th Annual Middle East Congress in Clinical Nutrition, May 11-13th, 2018 Athens, Greece

Title: The role of dietary Ω 3 fatty acids in paediatric asthma.

Papamichael Maria Michelle¹; Katsardis Charis³; Koutsilieris Michael³; Tsoukalas Dimitris⁴ ;
Lambert Katrina²; Erbas Bircan² ; Itsiopoulos Catherine¹

¹ *La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.*

² *La Trobe University, Department of Public Health, Melbourne, Australia*

³ *National & Kapodistrian University of Athens, Athens, Greece*

⁴ *European Institute of Nutritional Medicine, Rome, Italy*

ABSTRACT

Background

There is considerable interest in the use of omega 3 fatty acids in inflammatory diseases including asthma due to anti-inflammatory and immunomodulating effects.

Aim

We conducted a parallel randomized controlled trial of six months duration to investigate the effect of fatty fish (Ω 3) intake in paediatric asthma.

Methods

Seventy 72 children (54.2% boys; 45.8% girls), 5-12 years old with ‘mild asthma’ were selected from an asthma clinic in Athens, Greece and randomized to two groups. The intervention group consumed two fatty fish meals per week (≥ 150 g fillet fatty fish/meal) as part of the Mediterranean dietary pattern and the control group, their usual diet. Pulmonary function was assessed using spirometry and bronchial inflammation with exhaled Nitric Oxide analysis (eNO). Parents completed questionnaires to collect information regarding socio-demographics, medical history, asthma control, quality of life and dietary habits on behalf of their children. Adherence to the Mediterranean diet was assessed using the KIDMED score.

Results

A statistically significant change in eNO was observed in the intervention group (95%CI: -27.39, -0.91; beta = -14.15; p=0.037) after adjusting for confounders such as age, sex, regular physical activity and BMI. One unit increase in fatty fish intake reduced bronchial inflammation by 14 ppb for children in the intervention group. No significant differences were observed for spirometry.

Conclusion

The present study suggests that dietary Ω 3 fatty acids consumed as fatty fish might have a protective effect on bronchial inflammation in asthmatic children. Future clinical studies are recommended to replicate and confirm our findings.

12. World Congress on Nutrition & Dietetics, June 18-19 2018, Paris France.

Papamichael Maria Michelle et al., J Clin Nutr Diet 2018 Volume: 4
DOI: 10.4172/2472-1921-C1-002

FATTY FISH ($\Omega 3$): IS A DIET THERAPY FOR PAEDIATRIC ASTHMA?

Papamichael Maria Michelle¹, Katsardis Charis², Koutsilieris Michael², Tsoukalas Dimitris³, Lambert Katrina¹, Erbas Bircan¹ and Itsiopoulos Catherine¹

¹La Trobe University, Australia

²National & Kapodistrian University of Athens, Athens, Greece

³European Institute of Nutritional Medicine, Rome, Italy

Allergic disease can be referred to as the new mastiga of the 21st century. Childhood asthma is the most common respiratory disorder worldwide associated with increased morbidity, sometimes early mortality and decreased quality of life. Even then, the advances in pharmacotherapy, asthma control in children is poor. Hence, there is an urgent need for an alternative therapy that reduces burden of this disease. There is growing interest in omega-3 fatty acids in this disease due to anti-inflammatory and immunomodulating properties. However, their efficacy in asthma is controversial. The purpose of this study was to investigate the impact of fatty fish consumption in asthmatic children.

Methods: This was a six-month parallel randomized controlled trial. We selected 72 children (54.2% boys; 45.8% girls), 5-12 years old with doctor-diagnosed 'mild asthma' from an asthma clinic in Athens, Greece. Participants were equally randomized to two groups: the intervention group consumed two fatty fish meals per week (≥ 150 g fillet fatty fish/meal) as part of the Mediterranean dietary pattern and the control group, their usual diet. Pulmonary function was assessed using spirometry and exhaled Nitric Oxide analysis (eNO); asthma control and quality of life by questionnaires.

Results: At six months, we had 89% (64/72) participation rate. Multiple linear regression model showed a significant change in eNO for the intervention group (95% CI: -27.39, -0.91; beta=-14.15; p=0.037) after adjusting for confounders such as age, sex, regular physical activity and BMI. A unit increment in fatty fish consumption decreased lung inflammation by 14 ppb for the intervention group as compared to the control. No differences were observed for spirometry parameters, asthma control or quality of life scores.

Conclusion: The current study suggests that a healthy diet including two fatty fish meals per week should be included in asthma dietary guidelines.

Biography

Maria Papamichael is a Registered Dietician/Sports Nutritionist who has dedicated her life in educating people the importance of good nutrition and exercise in the prevention and management of disease as well as in improving health and well-being. Being an asthma sufferer since childhood, has motivated her to undertake a PhD research project at La Trobe University (Australia) to investigate the prophylactic potential of a Mediterranean diet enriched with fatty fish in the management of asthma in children.

sassipap@hotmail.com

13. 17th American Pediatric HealthCare & Infectious Diseases Congress June 27-28, 2018, Vancouver, Canada

Title: The prophylactic potential of fatty fish consumption on airway inflammation in childhood asthma

Papamichael Maria Michelle¹; Katsardis Charis³; Koutsilieris Michael³; Tsoukalas Dimitris⁴; Lambert Katrina²; Erbas Bircan²; Itsiopoulos Catherine¹

¹ *La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.*

² *La Trobe University, Department of Public Health, Melbourne, Australia*

³ *National & Kapodistrian University of Athens, Athens, Greece*

⁴ *European Institute of Nutritional Medicine, Rome, Italy*

ABSTRACT

According to the World Health Organization, 235 million people worldwide suffer from asthma. It is the most common chronic disease among children responsible for hospitalization, emergency visits, 10 million missed school days per year, impacts the productivity of working parents and inflicts a societal economic burden due to increased medical costs. Although genetic, environmental and epigenetic factors have been identified, an effective therapeutic intervention is yet to be identified. We conducted a single-centred parallel randomized controlled trial of six months duration to examine the prophylactic potential of dietary omega 3 fatty acid intake in pediatric asthma. Seventy-two children (54.2% boys; 45.8% girls), 5-12 years old with physician-diagnosed 'mild asthma' were selected from a paediatric clinic in Athens, Greece and randomized to two groups. The intervention group consumed two fatty fish meals per week (≥ 150 g fillet fatty fish/meal) and the control group, their usual diet. Pulmonary function was assessed using spirometry, bronchial inflammation with exhaled Nitric Oxide analysis (eNO), asthma control and quality of life by questionnaires. Multiple linear regression model showed a statistically significant change in eNO in the intervention group (95%CI: -27.39, -0.91; beta = -14.15; p=0.037) adjusting for confounders of age, sex, regular physical activity and BMI. Fatty fish intake twice weekly reduced bronchial inflammation by 14 ppb. No differences were observed for spirometry, asthma control or quality of life scores. This study suggests that two meals of fatty fish per week (≥ 150 g/ meal), a rich source of $\Omega 3$ fatty acids, might be an effective therapeutic intervention targeting inflammation in paediatric asthma.

14. 14th International Congress on Advances in Natural Medicines, Nutraceuticals & Neurocognition, July 19-20, 2018, London, UK

Title: Omega 3 fatty acids- A new therapeutic target for childhood asthma?

Papamichael Maria Michelle¹; Katsardis Charis³; Koutsilieris Michael³; Tsoukalas Dimitris⁴; Lambert Katrina²; Erbas Bircan²; Itsiopoulos Catherine¹

¹ *La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.*

² *La Trobe University, Department of Public Health, Melbourne, Australia*

³ *National & Kapodistrian University of Athens, Athens, Greece*

⁴ *European Institute of Nutritional Medicine, Rome, Italy*

ABSTRACT

Asthma has been referred to as an umbrella of multifactorial diseases with similar clinical features including mast cell and eosinophil infiltration causing airway hyperresponsiveness, inflammation and airway obstruction that subsequently lead to symptoms of wheeze, cough, dyspnoea, tightness in the chest especially at night and early morning. It has been hypothesized that diets low in omega 3 fatty acids have contributed to the escalation in childhood asthma prevalence. We conducted a clinical trial of six months duration to investigate the effect of fatty fish ($\Omega 3$) intake in paediatric asthma. Seventy-two (72) children (54.2% boys; 45.8% girls), 5-12 years old with doctor-diagnosed 'mild asthma' were selected from a paediatric clinic in Athens, Greece and randomized to two groups. The intervention group consumed two fatty fish meals per week (≥ 150 g fillet fatty fish/meal) as part of the Greek Mediterranean diet and the control group, their usual diet. Pulmonary function was assessed using spirometry, bronchial inflammation with exhaled Nitric Oxide analysis (eNO), asthma control and quality of life qualitatively using scores. Multiple linear regression model showed a statistically significant change in eNO for the intervention group (95%CI: -27.39, -0.91; beta = -14.15; p=0.037) after adjusting for confounders of age, sex, regular physical activity and BMI. A unit increase in fatty fish intake reduced bronchial inflammation by 14 ppb. No significant differences were observed for spirometry, asthma control or quality of life. This clinical study highlighted that dietary $\Omega 3$ fatty acids intake as fatty fish might be a useful adjunct therapy for paediatric asthma.



February 25-26, 2019
Prague, Czech Republic

3rd World Congress on
**Nutrition, Dietetics
and Nutraceuticals**

Maria M Papamichael et al., J Clin Nutr Diet 2019, Volume: 5
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THE IMPORTANCE OF VITAMIN D STATUS ON LUNG FUNCTION IN ASTHMATIC CHILDREN

**Maria M Papamichael¹, Katrina Lambert¹, Dimitris Tsoukalas³,
Michael Koutsilieris², Charis Katsardis², Bircan Erbas¹ and
Catherine Itsiopoulos¹**

¹La Trobe University, Australia
²National and Kapodistrian University of Athens, Greece
³European Institute of Nutritional Medicine, Italy

Vitamin D is a potent immunomodulator capable of dampening inflammatory signals in cells involved in the allergic response including asthma. However, evidence supporting a link between low vitamin D levels in children and higher risk of asthma exacerbations and poor lung function is inconsistent. The objective of this clinical trial was to investigate the potential role of vitamin D status in asthmatic children following a Mediterranean diet intervention. Greek asthmatic children aged 5-12 years old (51.6% male, 48.4% girls) were recruited from a pediatric asthma clinic in Athens, Greece and randomized into two groups. The intervention group consumed a Mediterranean diet plus two meals of fatty fish per week (≥ 150 g cooked filleted fish/meal) for six months and the control, their usual diet. Pulmonary function and bronchial inflammation were assessed using spirometry and Fractional exhaled Nitric Oxide analysis (FeNO). Serum vitamin D status was measured using Enzyme-Linked Immuno Assay (ELISA). Vitamin D deficiency was defined as serum 25-OH D < 25 ng/mL. At baseline, 64% of children (61% girls, 39% boys) were deficient in vitamin D. Multiple linear regression model adjusted for confounding factors of age, sex, regular physical activity and BMI showed that children in the intervention group with sufficient plasma vitamin D levels at baseline had increased FEV1/FVC by 4.89 units ($\beta = 4.89$; 95% CI: 1.19-8.61; $p = 0.013$) and FEF25-75% by 12.83 units ($\beta = 12.83$; 95% CI: 4.27-21.40; $p = 0.006$) as compared to the control group. No associations were observed for children deficient in vitamin D or for FeNO. In conclusion, consumption of Mediterranean diet supplemented with fatty fish significantly improved pulmonary function in asthmatic children with plasma vitamin D levels ≥ 25 ng/mL. More intervention studies are recommended to support the promising findings and to further clarify the role of vitamin D status in the management of asthma in children.

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16. 20th International Congress on Nutrition and Health, Mar 28-30, 2019, Stockholm, Sweden.

Title: The synergistic effect of vitamin D in pediatric asthma

Maria M. Papamichael¹; Katrina Lambert²; Dimitris Tsoukalas⁴; Michael Koutsilieris³; Charis Katsardis³; Bircan Erbas²; Catherine Itsiopoulos¹.

¹ La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Human Nutrition & Sport, Melbourne, Australia.

² La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy

ABSTRACT

Statement of Problem: Vitamin D deficiency and asthma prevalence in children are an emerging public health problem. Vitamin D has been demonstrated to possess potent immunomodulatory effects, including effects on T and B cells and increasing production of antimicrobial peptides that could lead to asthma specific beneficial effects. We sought to examine the effect of plasma vitamin D status in asthmatic children consuming a Mediterranean diet enriched with fatty fish.

Methodology: This study was a single-centred randomized controlled trial of six months duration. Asthmatic children, 5-12 years old (51.6% male), attending a pediatric asthma clinic in the greater city of Athens, Greece were randomized into intervention versus control groups. The intervention group was instructed to follow the Greek Mediterranean dietary pattern and to include two meals of fatty fish weekly (≥ 150 g filleted cooked fish/meal) for a period of six months. In comparison, the control group, their usual diet. Asthma status was measured using spirometry and exhaled Nitric Oxide analysis (eNO). Enzyme-Linked Immuno Assay was used to determine serum vitamin D concentrations with vitamin D sufficiency defined as plasma 25-OH D ≥ 25 ng/mL. **Findings:** Multiple linear regression model revealed that FEV₁/FVC increased by 4.89 units ($\beta = 4.89$; 95%CI: 1.19- 8.61; $p = 0.013$) and FEF_{25-75%} by 12.83 units ($\beta = 12.83$; 95%CI: 4.27-21.40; $p = 0.006$) in the

intervention group with sufficient plasma vitamin D levels at baseline as compared to the control group after adjusting for confounders of age, sex, regular physical activity and BMI. No associations were observed for children deficient in plasma vitamin D or for eNO.

Conclusion & Significance: These findings suggest a synergistic effect of plasma vitamin D on pulmonary function in asthmatic children consuming a Mediterranean diet enriched with fatty fish. Normalization of vitamin D status in asthmatic children could alleviate symptoms and reduce asthma burden.

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3. Cashman KD et al, 2016. Vitamin D deficiency in Europe: pandemic? *Am J Clin Nutr.* 2016 Apr;103(4):1033-44. doi: 10.3945/ajcn.115.120873.
4. Kerley CP et al, 2015. Vitamin D as an adjunctive therapy in asthma. Part 1 : A review of potential mechanisms. *Pulm Pharmacol Ther.* 2015 32: 60-74. doi: 10.1016/j.pupt.2015.02.004.
5. Ali N S & Nanji K, 2017 A Review on the Role of Vitamin D in Asthma. *Cureus* 9(5): e1288. doi:10.7759/cureus.1288.

17. World Summit on Pediatrics June 20-23 2019, Berlin, Germany

Title: Precision medicine advances in childhood atopic diseases: Results from atopic dermatitis and asthma

Dimitris Tsoukalas¹, Gerasimos Tsilimidos¹, **Maria Michelle Papamichael**², Charis Katsardis⁴, Bircan Erbas³, Catherine Itsiopoulos².

¹*European Institute of Nutritional Medicine, E.I.Nu.M.*

²*La Trobe University, School of Allied Health, Human Services & Sport, , Department of Dietetics, Nutrition & Sport, Melbourne, Australia.*

³*La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia*

⁴*National & Kapodistrian University of Athens, Athens, Greece*

Introduction: Atopic dermatitis (AD) and asthma are common childhood atopic diseases that occur due to immune system dysregulation and manifest as allergic inflammation. 10-20% of children in US and Western Europe have atopic dermatitis and are treated with anti-inflammatory steroid creams. Asthma is the most common chronic respiratory disease in children worldwide affecting 1-20% of the child population. Therapeutic approaches include inhaled treatment or pills to reduce inflammation and facilitate breathing. The etiopathogenesis of atopic diseases includes genetic factors and environmental triggers. Currently, the diagnosis of atopic diseases can be difficult due to the lack of useful markers of asthma in a clinical setting. Precision medicine integrates data from genome, microbiome, dietary and lifestyle habits to study the human body as a whole.

Objective:

To identify the underlying cause of pediatric asthma and atopic dermatitis and manage the triggers of the disease to improve clinical symptoms.

Materials and Methods:

Targeted Metabolomic analysis and subsequent personalized treatment were performed in 30 Korean infants diagnosed with (AD), that did not respond to standard therapeutic actions and 72 children with mild asthma. Metabolomics was performed in blood and urine samples using Gas chromatography-Mass Spectrometry and personalized treatment included nutritional intervention to restore the deficiencies in nutrients and biochemical disruptions. In the case of asthma, the pulmonary function was assessed using spirometry and bronchial inflammation by fractional exhaled nitric oxide analysis. For atopic dermatitis skin lesions were assessed before and after treatment (medical pictures).

Results:

Metabolomic analysis of children with AD revealed significant metabolic disruption in Citric Acid Cycle compatible with mitochondrial dysfunction due to xenobiotics toxicity, lack of the amino-acid glutamine and ubiquinol, cytochrome C dysfunction, and imbalances in selected fatty acids markers. The metabolomic analysis of mild asthmatic children showed a strong association between key metabolic markers and pulmonary function measurements. Restoration of specific nutrient deficiencies and personalized diet based on the metabolomic analysis resulted in drastic improvement of the skin lesions and lung function respectively, within a few weeks from start of treatment in most cases.

Conclusions:

We demonstrated that diet and lifestyle had a determinant role on the disease progression and highlighted the potency of metabolomics in identifying the nutritional deficiencies in the disease state.

18. 28th International Conference on Pediatrics Health Aug 12-13, 2019 Rome, Italy

Title: Urinary metabolomic profile of Greek asthmatic school-children

Maria Michelle Papamichael¹; Charis Katsardis²; Dimitris Tsoukalas³; Bircan Erbas⁴; Catherine Itsiopoulos^{1,5}

¹ La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Nutrition & Sport, Melbourne, Australia.

² National & Kapodistrian University of Athens, Athens, Greece

³ European Institute of Nutritional Medicine, Rome, Italy

⁴ La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia

⁵ Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia.

Abstract

In clinical practice, biomarkers can provide complementary information to conventional pulmonary tests, symptoms, spirometry, exhaled nitric oxide, PC20methacholine and histamine. They are useful in establishing diagnosis, monitoring of the disease progression and in response to treatment. The development of non-invasive sampling methods and detection techniques for the identification of components involved in airway inflammation including the determination of biomarkers, would greatly contribute to our current insight in airway inflammation associated with various asthma phenotypes as well as to customize individually-targeted therapies. In children, reliable, non-invasive biomarkers would be valuable. We applied metabolomic analysis to study the association between urinary organic acid concentrations and pulmonary function in 72 Caucasian children (5-12 years old) suffering with asthma determined by gas chromatography and mass spectrometry. Pulmonary function was assessed by spirometry, exhaled Nitric Oxide (eNO) and Asthma Control Questionnaire. Targeted metabolomic analysis identified 34 unique urinary organic acids in children. Gender differences were observed for asthma control ($p=0.02$) and lactic acid ($p=0.03$). Correlations were found between lactic acid and Forced Expiratory Volume in 1 second (FEV_1) ($p=0.02$), Forced Vital Capacity (FVC) ($p=0.03$); 4-hydroxyphenylacetic acid and FEV_1 ($p=0.01$), FVC ($p=0.01$); 5-hydroxyindoleacetic acid and FEV_1/FVC ($p=0.03$), eNO ($p=0.05$); glycolic acid with Peak Expiratory Flow (PEF) ($p=0.03$); malic, glutaric and 2-hydroxyisobutyric acids with asthma control ($p=0.01$; 0.05 ; 0.02 respectively). In conclusion, biomarkers such as urinary organic acid can be useful tools in clinical practice for the diagnosis and management of asthma in children as well as in proposing novel therapeutic targets.

Keywords: Asthma, urinary organic acids, pulmonary function, children, spirometry

Title: Overweight/obesity increases ventilatory capacity and reduces FeNO in asthmatic children

Maria Michelle Papamichael¹; Charis Katsardis³; Dimitris Tsoukalas⁴; Bircan Erbas²; Catherine Itsiopoulos^{1,5}

¹La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Human Nutrition & Sport, Melbourne, Australia.

² La Trobe University, School of Psychology and Public Health, Department of Public Health, Melbourne, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy

⁵ Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia.

Abstract

Statement of Problem: Universally, bronchial asthma is the most common chronic respiratory disorder in children characterised by airway inflammation, bronchial hyperresponsiveness and recurrent episodes of reversible airway obstruction. Excess weight represents a major global health challenge because of adverse health-outcomes including asthma. The concurrent rise in asthma and pediatric obesity postulate a possible link between the two conditions. Excess weight may impact asthma via multiple mechanisms including pulmonary mechanics, lifestyle, dietary, immunological, hormonal and common genetic factors. High bodyweight was found to be associated with reduced pulmonary function and Fractional exhaled Nitric Oxide (FeNO) in adult subjects. Yet the current literature focusing on the effect of obesity and overweight on lung function and FeNO in asthmatic children remains controversial. The *objective* of this study was to investigate the effect of excess weight on pulmonary function and exhaled nitric oxide in a sample of 72 Greek asthmatic children (5-12 years old) participating in a Mediterranean diet and childhood asthma intervention study. **Methodology:** Pulmonary function was assessed using spirometric measures (FEV₁, FVC, FEV₁/FVC, PEF, FEF_{25-75%}) and eosinophilic bronchial inflammation by FeNO. Body Mass Index (weight/height²) was used to measure excess bodyweight and categorized using age- and sex-specific BMI cut-off values for ages 2-18 years as proposed by the International Obesity

Task Force (normal weight (≥ 17 and < 25 kg/m²), overweight (≥ 25 and < 30 kg/m²), and obese (≥ 30 kg/m²). **Findings:** Data analysis showed a positive linear relationship between BMI and FVC ($p=0.01$) and FEV₁ ($p=0.03$). FeNO was lower in the overweight/obese group as compared to normal weight ($p=0.03$). **Conclusion & Significance:** High BMI in asthmatic children was associated with increased lung volume (FVC) and airflow (FEV₁) along with reduced FeNO. Future research is needed to establish whether disproportionate lung growth and non-eosinophilic bronchial inflammation might be the underlying mechanisms for this paradox.

Recent Publications:

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4. Tantisira KG, Weiss ST (2001) Childhood infections and asthma: at the crossroads of the hygiene and Barker hypotheses. Respir Res 2(6): 324–7.
5. Yao T-C, Tsai H-J, Chang S-W, Chung R-H, Hsu J-Y, Tsai M-H, et al. (2017) Obesity disproportionately impacts lung volumes, airflow and exhaled nitric oxide in children. PLoS ONE 12(4): e01746.

20. 15th PanHellenic Nutrition & Dietetics Conference Dec 13-15, 2019 Athens, Greece

DOES A MEDITERRANEAN DIET ENRICHED WITH FATTY FISH IMPACT LUNG FUNCTION IN CHILDREN WITH ASTHMA?

Maria Michelle Papamichael; Charis Katsardis; Katrina Lambert; Dimitris Tsoukalas; Bircan Erbas; Catherine Itsiopoulos.

¹La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Human Nutrition & Sport, Melbourne, Australia.

² La Trobe University, School of Psychology and Public Health, Department of Public Health, Melbourne, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴European Institute of Nutritional Medicine, Rome, Italy

⁵ Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia.

ABSTRACT**Introduction**

The Mediterranean diet a sustainable dietary pattern rich in foods with anti-inflammatory potential has multiple health benefits. However, it is unknown whether the Mediterranean diet reduces asthma burden in children.

Purpose We conducted a six-month randomized controlled trial to investigate the efficacy of the Mediterranean diet on airway inflammation in asthmatic children.

Methods & Materials

Seventy-two (72) Greek asthmatic patients (5-12 years) were recruited from a pediatric asthma clinic in Athens and randomized to intervention versus control groups. The intervention group consumed a Mediterranean diet enriched with 2 fatty fish meals/week (≥ 150 g cooked fillet/meal) for six months; the control group, their habitual diet. Lung function was assessed using spirometry; airway inflammation via Fractional exhaled Nitric Oxide (FeNO) and Mediterranean diet compliance using the KIDMED score.

Results

Participation rate was 89% (64/72), 52% were boys, mean age 8 years, and only 17% of children reported strong adherence to the Mediterranean diet (KIDMED score of 8-12). Fish intake increased from 13% to 84% in the intervention group supported by a 120% increase in serum DHA. Compared to the control, FeNO decreased by 14 ppb from baseline in the intervention group in a linear regression model controlled for age, gender, regular physical activity and BMI ($p=0.04$; $\beta=-14.15$ ppb; 95% CI: -27.39, -0.91). No difference in spirometry was observed between groups.

Conclusion

A Mediterranean diet enriched with omega-3 fatty acids derived from fatty fish significantly reduced airway inflammation in asthmatic children. Targeting airway inflammation may reduce asthma burden in children.

20. 25th Hellenic Nutrition & Dietetics Conference Dec 13-15, 2019 Athens, Greece

Title Does a Mediterranean diet enriched with fatty fish impact lung function in asthmatic children? (Greek translation)

ΜΠΟΡΕΙ ΜΙΑ ΜΕΣΟΓΕΙΑΚΗ ΔΙΑΤΡΟΦΗ ΕΜΠΛΟΥΤΙΣΜΕΝΗ ΜΕ ΛΙΠΑΡΟ ΨΑΡΙ ΝΑ ΕΠΗΡΕΑΣΕΙ ΤΗΝ ΠΝΕΥΜΟΝΙΚΗ ΛΕΙΤΟΥΡΓΙΑ ΣΤΑ ΑΣΘΜΑΤΙΚΑ ΠΑΙΔΙΑ;

Μαρία Μισέλ Παπαμιχαήλ¹, Χάρης Κατσαρδής³, Katrina Lambert ², Δημήτρης Τσουκαλάς ⁴, Bircan Erbas ², Κατερίνα Ιτσιόπουλος ^{1,5}.

¹ Πανεπιστήμιο La Trobe, Σχολή Επιστημών Υγείας, Μελβούρνη, Αυστραλία.

² Πανεπιστήμιο La Trobe, Σχολή Δημόσιας Υγείας, Μελβούρνη, Αυστραλία.

³ Εθνικό & Καποδιστριακό Πανεπιστήμιο Αθήνας, Ελλάδα

⁴ Ευρωπαϊκού Ινστιτούτο Διατροφικής Ιατρικής, Ρώμη, Ιταλία

⁵ Πανεπιστήμιο Murdoch, Σχολή Επιστημών Υγείας, Μηχανικών και Εκπαίδευσης, Πέρθ, Αυστραλία

ΠΕΡΙΛΗΨΗ

Εισαγωγή

Η Μεσογειακή διατροφή είναι πλούσια σε τρόφιμα με αντιφλεγμονώδη δράση.

Σκοπός

Διενεργήσαμε εξάμηνη τυχαιοποιημένη ελεγχόμενη δοκιμή για να διερευνήσουμε την αποτελεσματικότητα της Μεσογειακής διατροφής στη βρογχική φλεγμονή στα ασθματικά παιδιά.

Μεθοδολογία/Υλικά

Εβδομήντα δύο Ελληνόπουλα με άσθμα (5-12 ετών) από μια παιδιατρική κλινική άσθματος στην Αθήνα τυχαιοποιήθηκαν σε ομάδες παρέμβασης έναντι ελέγχου. Η ομάδα παρέμβασης κατανάλωνε μια Μεσογειακή διατροφή εμπλουτισμένη με 2 γεύματα λιπαρού ψαριού/ εβδομάδα (≥ 150 γρ μαγειρεμένο φιλέτο/γεύμα) για έξι μήνες και η ομάδα ελέγχου, τη συνήθη διατροφή της. Η πνευμονική λειτουργία αξιολογήθηκε με σπιρομέτρηση. Η βρογχική φλεγμονή με τη μέτρηση του εκπνεόμενου μονοξειδίου του αζώτου (FeNO) και της συμμόρφωσης στη Μεσογειακή διατροφή μέσω της βαθμολογίας KIDMED.

Αποτελέσματα

Το ποσοστό συμμετοχής ήταν 89% (64/72), το 52% ήταν αγόρια με μέση ηλικία 8 ετών και μόνο το 17% των παιδιών ανέφερε υψηλή προσκόλληση στη Μεσογειακή διατροφή (βαθμολογία KIDMED 8-12). Η πρόσληψη ψαριών αυξήθηκε από 13% σε 84% στην ομάδα παρέμβασης και υποστηρίχθηκε από την αύξηση του DHA ορού κατά 120%. Σε σύγκριση με την ομάδα ελέγχου, το FeNO μειώθηκε κατά 14 ppb από την αρχική τιμή στην ομάδα παρέμβασης σε ένα μοντέλο γραμμικής παλινδρόμησης προσαρμοσμένο για την ηλικία, το φύλο, τη φυσική δραστηριότητα και το ΔΜΣ ($p = 0.04$, $\beta = -14.15$ ppb, 95% CI: -27.39, -0.91). Δεν παρατηρήθηκε διαφορά στη σπιρομέτρηση μεταξύ των ομάδων.

Συμπέρασμα

Μια Μεσογειακή διατροφή εμπλουτισμένη με ωμέγα-3 λιπαρά οξέα από λιπαρά ψάρια μείωσε σημαντικά τη βρογχική φλεγμονή στα ασθματικά παιδιά. Η στόχευση στη βρογχική φλεγμονή μπορεί να μειώσει την επιβάρυνση του άσθματος στα παιδιά.

21. Annual Conference of the College of Pediatrics Feb 14-15th, 2020, Athens, Greece

THE INFLUENCE OF BODY WEIGHT ON LUNG FUNCTION IN ASTHMATIC CHILDREN

Papamichael Maria Michelle¹; Tsoukalas Dimitris⁴; Erbas Bircan²; Itsiopoulos Catherine^{1,5}; Katsardis Charis³

¹ La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Human Nutrition & Sport, Melbourne, Australia

² La Trobe University, School of Psychology and Public Health, Department of Public Health, Melbourne, Australia

³ National and Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy

⁵ Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia

Abstract

Introduction

Overweight represents a major global health challenge because of detrimental health outcomes. Whether overweight affects asthma in children is controversial.

Aim

To investigate the effect of excess weight on pulmonary function in pediatric asthma.

Materials/Methods

In this study participated 72 Greek children with mild asthma from a pediatric asthma clinic in Athens (5-12 years old; 54.2% boys). Pulmonary function was assessed using spirometry (FEV₁, FVC, FEV₁/FVC, PEF, FEF_{25-75%}) and eosinophilic bronchial inflammation measured by Fractional exhaled Nitric Oxide (FeNO). Body Mass Index (weight/height²) was used to measure excess bodyweight and categorized using International Obesity Task Force cut-offs (normal weight (≥ 17 and < 25 kg/m²), overweight (≥ 25 and < 30 kg/m²), and obese (≥ 30 kg/m²)).

Results

Positive correlations were observed between BMI and FVC ($p=0.013$) and FEV₁ ($p=0.026$). After stratification to overweight/obese versus normal weight, FeNO was lower in the overweight/obese group as compared to normal weight (median 5 vs 11 ppb respectively, $p=0.027$). In the regression model there was a positive association of overweight/obese on FEF_{25-75%} as compared to normal weight adjusted for age, ht, sex, regular physical activity, medication and Mediterranean diet adherence ($p=0.043$; $\beta=11.65$ units, 95% CI: 0.36- 22.94). No effect of BMI on FeNO was observed.

Conclusions

High BMI in asthmatic children might influence pulmonary function. Further research is recommended to confirm whether this is caused by disproportionate lung growth (dysanapsis) and non-eosinophilic bronchial inflammation.

THE INFLUENCE OF BODY WEIGHT ON LUNG FUNCTION IN ASTHMATIC CHILDREN (Greek translation)

Τίτλος: Η ΕΠΙΔΡΑΣΗ ΤΟΥ ΣΩΜΑΤΙΚΟΥ ΒΑΡΟΥΣ ΣΤΗΝ ΠΝΕΥΜΟΝΙΚΗ ΛΕΙΤΟΥΡΓΙΑ ΤΩΝ ΑΣΘΜΑΤΙΚΩΝ ΠΑΙΔΙΩΝ

Παπαμιχαήλ Μαρία Μισέλ¹; Τσουκαλάς Δημήτρης⁴; Erbas Bircan²; Ιτσιόπουλος Κατερίνα^{1,5}; Κατσαρδής Χαράλαμπος³

¹ Πανεπιστήμιο Λα Τρομπ, Τμήμα Επιστήμης Διαιτολογίας-Διατροφής, Μελβούρνη, Αυστραλία

² Πανεπιστήμιο Λα Τρομπ, Τμήμα Ψυχολογίας και Δημόσια Υγείας, Μελβούρνη, Αυστραλία

³ Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα, Ελλάδα

⁴ Ευρωπαϊκό Ινστιτούτο Διατροφικής Ιατρικής Ρώμη, Ιταλία

⁵ Πανεπιστήμιο Murdoch, Σχολή Επιστήμης Υγείας, Μηχανικών και Εκπαίδευσης Πέρθ, Αυστραλία.

ΠΕΡΙΛΗΨΗ

Εισαγωγή

Το υπερβολικό βάρος αποτελεί μια σημαντική παγκόσμια πρόκληση για την υγεία λόγω των επιβλαβών επιπτώσεων στην υγεία. Αν το υπερβολικό βάρος επηρεάζει το άσθμα στα παιδιά παραμένει αμφιλεγόμενο.

Σκοπός

Να διερευνηθεί η επίδραση του υπερβολικού βάρους στην πνευμονική λειτουργία στο παιδικό άσθμα.

Υλικό/Μέθοδος

Στην μελέτη έλαβαν μέρος 72 Ελληνόπουλα από παιδοπνευμονολογικό ιατρείο της Αθήνας (ηλικίας 5-12 χρόνων, 54,2% αγόρια) με ήπιο άσθμα. Η πνευμονική λειτουργία αξιολογήθηκε με σπιρομέτρηση (FEV₁, FVC, FEV₁/FVC, PEF, FEF_{25-75%}) και η ηωσινοφιλική βρογχική φλεγμονή με τη μέτρηση του κλασματικού εκπνεόμενου μονοξειδίου του αζώτου (FeNO). Ο Δείκτης Μάζας Σώματος (ΔΜΣ) (βάρος / ύψος²) χρησιμοποιήθηκε για τη μέτρηση του υπερβολικού σωματικού βάρους και ταξινομήθηκε σύμφωνα με τη Διεθνή Ομάδα Παχυσαρκίας (IOTF) (φυσιολογικό 17-25 kg/m², υπέρβαρο 25-30 kg/m² και παχύσαρκο ≥ 30 kg/m²).

Αποτελέσματα

Παρατηρήθηκαν θετικές συσχετίσεις μεταξύ ΔΜΣ και FVC (p=0.013) και FEV₁ (p=0.026). Μετά το διαχωρισμό σε υπέρβαρους/ παχύσαρκους έναντι ομάδας φυσιολογικού βάρους, το FeNO ήταν χαμηλότερο στους υπέρβαρους/παχύσαρκους σε σύγκριση με το φυσιολογικό βάρος (διάμεσο FeNO 5 έναντι 11 ppb αντίστοιχα· p=0.027). Στο μοντέλο παλινδρόμησης παρατηρήθηκε θετική συσχέτιση των υπέρβαρων/ παχύσαρκων στο FEF_{25-75%} σε σύγκριση με το φυσιολογικό βάρος, προσαρμοσμένα σε ηλικία, φύλο, φυσική δραστηριότητα, ΔΜΣ, φαρμακευτική αγωγή και συμμόρφωση στη Μεσογειακή διατροφή (p=0.043; β =11.65 μονάδες, 95% CI: 0.36- 22.94). Δεν παρατηρήθηκε καμία επίδραση του ΔΜΣ στο FeNO.

Συμπέρασμα

Ο υψηλός ΔΜΣ στα ασθματικά παιδιά μπορεί να επηρεάζει την πνευμονική λειτουργία. Απαιτείται περαιτέρω έρευνα για να διαπιστωθεί αν οφείλεται σε δυσανάλογη ανάπτυξη πνευμόνων και διαμέτρου των αεραγωγών (δυσάναψη) και στη μη ηωσινοφιλική φλεγμονή.

Posters

1.1st World Conference on the Mediterranean diet 6-8 July, 2016 Milano, Italy



The prophylactic potential of a Mediterranean dietary pattern enriched with fatty fish in asthmatic children: A Randomized Controlled Trial

M. V. Papamichail, PhD Cand.; Assoc. Prof. Dr. C. Fotopoulou, PhD
Mediterranean Diet Research Group
La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia



Background: Asthma has rapidly become the most frequent chronic disease in children globally, placing significant disease burden as the most common reasons for hospitalisation, absence from school and work for sufferers and their parents/caregivers, respectively (WHO, 2013; GINA, 2012). Managed primarily symptomatically with medications as there is no known cure. Emerging evidence indicates that diet and lifestyle play a role in the aetiology and management, with potential for a protective effect of a Mediterranean diet (Arvaniti et al., 2011; Chatzi et al., 2009). In particular, fatty fish consumption has been associated with improved pulmonary function and reduced symptoms in asthmatic children (Hodge et al., 2008; Tabek et al., 2008). But clinical trials are lacking.

Aim: to investigate whether fatty fish consumption as part of the Greek Mediterranean dietary pattern improves pulmonary function and decreases symptoms in asthmatic children.

Primary Hypotheses:

Does fatty fish consumption?

- improve respiratory function
- decrease asthma episodes,
- day and night symptoms
- decrease days hospitalized/medical care
- decrease need for medication
- decrease days absent from school
- improve quality of life

Assessment Tools:

Pulmonary function: Spirometry (FEV1), doctor's/parents report of symptoms, number of days requiring hospitalization/medical care, days absent school, need for medication

Asthma Control: Child Asthma Control Test (CACT) (Schatz, 2006)

Quality of Life: Pediatric Asthma Quality of Life (PAQOL) Questionnaire (Juniper, 1998)

Dietary habits: Food Frequency Questionnaire validated for Greek children (Antonopoulou et al., 2013)

Adherence to Mediterranean Diet: KIDMED SCORE (Serra-Majem et al., 2004)

Biomarkers: Plasma fatty acid composition, antioxidant levels, inflammatory markers.

Outcome measurements baseline, 3 and 6 months

Sample Size Calculation: G Power Analysis (3.1), 80% power, medium effect size= 0.4, 5% significance level and allowing for dropout rate 20% produced a total sample of 64 participants, i.e. 32 patients in each group (Lee et al., 2012)

Methods:

Study Design: parallel randomised controlled study

Duration: 6 months

Venue: Athens, Greece

Recruitment: Paediatric Asthma Clinic

Participants: asthmatic children aged 5-12 years

Intervention: type: dietary

Randomized into 2 groups: intervention v control

Intervention group: Consumption of two fatty fish meals (150g cooked) per week as part of Greek Mediterranean Dietary pattern over a period of 6 months (Willet, Sacks & Trichopoulos, 1995; Protopapas, 2014)

Control group: consume their usual diet.

Applications: This study is important in identifying the potential of a Mediterranean diet supplemented with fatty fish in ameliorating asthma symptoms, improving pulmonary function and quality of life in children with asthma.

Clinical Trial Registration: [AC116N12618030492459p](https://clinicaltrials.gov/ct2/show/study?term=AC116N12618030492459p)

References:

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Conflict of interest: None

Contact: Maria Papamichail

E-mail: www.maria@ltschool.com

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LA TROBE UNIVERSITY **10th EFAD Conference Rotterdam, Sep 29-30, 2017**

"A randomized controlled trial on the impact of a Mediterranean diet enriched with fatty fish on asthma in Greek children: Study Protocol"

Papamichael Maria¹, Katsaris Christos², Tsoukalas Dimitrios¹, Ertas Sincan³, Tsiakopoulos Catherine⁴

¹Department of Rehabilitation, Nutrition & Sport, La Trobe University, Melbourne, Australia
²Department of Public Health, La Trobe University, Melbourne, Australia
³Department of Medicine, National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

Introduction: The asthma epidemic in children has become a major public health concern globally [1]. Studies have documented a correlation between diet [2, 3] and overweight [4] in childhood asthma prevalence. Emerging evidence from epidemiological studies indicates a potential prophylactic effect of the Mediterranean diet in childhood asthma [5] as well as fish intake due to anti-inflammatory and immune-modulating effects [6]. However, clinical trials in children are lacking and this warrants further investigation. **Aims:** to investigate whether fatty fish consumption as part of the Mediterranean diet reduces asthma symptoms in children.

Methodology

- Study design:** Parallel Randomized Controlled Trial
- Duration:** 6 months
- Setting:** private pediatric asthma clinic in Athens, Greece
- Population:** Greek Children aged 5-12 yrs with 'mild' asthma [1]
- Sample:** 72 patients (54.2% *at*, 45.8% *FF*)
- Mean age:** 10 ± 2 yrs
- Recruitment:** November 2016
- Randomized:** equally into 2 groups:
- Intervention group:** consumes a 150g fatty fish/week, twice weekly for 6 months
- Control group:** their usual diet

Assessment Tools

- Anthropometry:** weight, height, BMI [7]
- Pulmonary function:** Spirometry (FEV1), exhaled Nitric Oxide Analysis, parent report of symptoms [8]
- Asthma Control:** Asthma Control Questionnaire (ACQ) [9]
- Quality of Life (QoL):** Paediatric Quality of Life Questionnaire [9]
- Dietary habits:** FFQ [10]
- Adherence to Med diet:** KIDMED Score [11]
- Metabolic profile:** Biochemical tests
- Assessment time-points:** baseline & 6 months

Statistical Analysis

Clinical Trial No.: ACTRN126160009924009

Sample size calculation (G Power Analysis): 64 patients (32 per group)
 Data was analysed using SPSS (Version 20). Continuous variables were assessed for normality using graphical methods, descriptive statistics and Shapiro-Wilk's test. Differences between groups were analysed using t-Test for normally distributed variables and Mann-Whitney test in the case of non-normality. P-value was considered to be statistically significant at 5% level.

Baseline Results

Participation rate: 90% (65/72)

Table 1. Characteristics of sample

Variable	Gender	
	Girls (n=32)	Boys (n=33)
Age (mean ± SD)	10.6 ± 1.5	10.5 ± 1.5
Height (mean ± SD)	136.5 ± 14.3	132.0 ± 12.94
Weight (mean ± SD)	34.1 ± 12.3	33.8 ± 11.6
BMI (kg/m ²)	18.2 ± 3.62	19.4 ± 3.89

Anthropometry:
 • Height: 64% normal; 36% tall
 • Weight: 2.78% 'underweight', 57% 'normal weight', 20% 'overweight' and 11% 'obese' according to Hellenic Pediatric Growth Charts, 2015 [7]

Pulmonary functions:
 • Spirometry: FEV1 was normal (≥ 80% predicted)
 • eNO: No bronchial inflammation (eNO < 10ppb)
 • Asthma Control: 88.70% of children had 'well-controlled' asthma (score < 0.75), 0.50% 'not well-controlled' and 2.00% 'uncontrolled'
 • QoL: 74.50% of children 'No impairment' (score=7), 19.70% 'Hardly impaired' and 5.60% 'Very slightly impaired'

Table 2. Pulmonary function, asthma control, QoL per group

Pulmonary function	Group	P-value
Variable	Intervention	Control
Expected FEV1 (L)	51.0 (n=16)	50.1 (n=16)
Observed FEV1 (L)	31 (45%, 27.00)	9 (50%, 57.88)
eNO (ppb)	6.29 (0.14, 0.27)	8.29 (0.14, 0.44)
Asthma Control Score	6.92 (6.73, 7.00)	6.92 (6.82, 7.00)
QoL Score	6.92 (6.73, 7.00)	6.92 (6.82, 7.00)

Adherence Mediterranean diet

Fig 1. Children's adherence to the Mediterranean diet according to KIDMED score (11)
 From Fig. 1, 10.20% optimal adherence to Med diet pattern

Table 3. KIDMED score per intervention group at baseline

Group	Mean ± SD	P-value
Intervention	5.39 ± 2.80	0.97
Control	5.37 ± 2.06	

No statistical significant differences in anthropometry, pulmonary function, asthma control, QoL, biochemical tests, adherence to Med diet were observed between intervention groups or gender: *p* > 0.05

Conclusion: Future public health strategies should focus on promoting healthy eating, physical activity in the prevention of overweight/obesity and management of childhood asthma.

Notes: (1) [1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21] [22] [23] [24] [25] [26] [27] [28] [29] [30] [31] [32] [33] [34] [35] [36] [37] [38] [39] [40] [41] [42] [43] [44] [45] [46] [47] [48] [49] [50] [51] [52] [53] [54] [55] [56] [57] [58] [59] [60] [61] [62] [63] [64] [65] [66] [67] [68] [69] [70] [71] [72] [73] [74] [75] [76] [77] [78] [79] [80] [81] [82] [83] [84] [85] [86] [87] [88] [89] [90] [91] [92] [93] [94] [95] [96] [97] [98] [99] [100]

Contact details:
 Dr. Papamichael Maria
 maria.papamichael@latrobe.edu.au

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THE ROLE OF DIETARY Ω3 FATTY ACIDS IN PAEDIATRIC ASTHMA

Papamichael Maria Michelle¹; Katsaridis Charis¹; Koutsilieris Michael¹; Tsoukalas Dimitris¹; Lambert Katrina¹; Erbes Bircan²; Itsiopoulos Catherine¹

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Melbourne, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

Background/Aim

There is considerable interest in the use of omega 3 fatty acids in inflammatory diseases including asthma due to anti-inflammatory and immune-modulating effects [1-3]. However, the effectiveness of EPA/DHA in childhood asthma is unclear due to inconsistency among studies [4] and paucity of well-designed clinical trials [5]. Hence this warrants further investigation. We aimed to conduct a parallel randomized controlled trial of six months duration to investigate the effect of fatty fish (Ω3) intake in paediatric asthma.

Methods & Materials

► **Study design:** parallel single-centred randomized controlled trial [6]
 ► **Duration:** six months
 ► **Population:** 72 children, 5-12 years old
 ► **Sample:** 54% ♂ (n=30), 46% ♀ (n=32); Mean age 8±2 years old
 ► **Enrollment period:** November-December 31st, 2016.
 ► **Setting:** paediatric asthma clinic, Athens, Greece
 ► **Randomized to 2 groups:** Intervention (n=36) vs. Control (n=36)

► **Intervention:**
 1. **Intervention group:** consumed two fatty fish meals per week (x 150g filleted fatty fish/meal)⁷ as part of the Greek Mediterranean diet [8]
 2. **Control group:** consumed their usual diet.

► **Assessment Tools:**
 1. **Anthropometry:** Weight, height, BMI [9]
 2. **Pulmonary function:** Spirometry [6]
 3. **Branchial inflammation:** exhaled Nitric Oxide analysis (eNO) [6]
 4. **Questionnaires:** Socio-demographics, medical history
 5. **Asthma control:** Child Asthma Control Questionnaire [10]
 6. **Quality of Life:** Pediatric Asthma Quality of Life Questionnaire [11]
 7. **Dietary habits:** PANAACEA-FFD [12]
 8. **Adherence to Mediterranean diet:** ECODED tool [13]
 9. **Physical activity:** SAAC Environmental Questionnaire [14]
 10. **Biochemical tests:** fatty acid composition, organic acids, Vitamin D & dietary biomarkers using gas-chromatography and mass-spectrometry

► **Assessment time-points:** baseline & six months

*Fatty fish included sardines, salmon, mackerel, anchovies, trout, gilthead sea bream

Clinical Trial No: ACTRN12616000402409

Results

► We had 85% participation rate (64/72). Pulmonary function tests showed that children in both groups had normal lung and no bronchial inflammation. As for asthma control and quality of life scores, all children had 'well-controlled' asthma and 'no impairment' (Table 1).

Table 1. Baseline characteristics of sample per intervention group

Variable*	Total (n=72)	Intervention group (n=36)	Control group (n=36)	P-value
Age (years)	Mean: 8.00	8.00	8.00	0.63
Male n (%)	34 (47.2%)	17 (47.2%)	17 (47.2%)	0.99
Overweight/obese	35%			
Regular Physical Activity n (%)	35% (48.6%)	17 (47.2%)	18 (50.0%)	0.66
BRONIS-VIS	85% (26.8%)	17 (47.2%)	19 (52.8%)	0.81
Pulmonary Function:				
FVC (% predicted)	103.23	103.23	103.23	0.66
PVC (% predicted)	104.62	104.62	104.62	0.11
FEV1 (% predicted)	122.85	122.85	122.85	0.79
PEF (% predicted)	104.52	104.52	104.52	0.96
FEV1/FVC (% predicted)	102.28	102.28	102.28	0.66
Exhaled Nitric Oxide (ppb)	17.09	17.09	17.09	0.14
AQOL score	0.15	0.15	0.15	0.96
QOL score	4.77	4.77	4.77	0.93
ECODED score	5.32	5.32	5.32	0.93
Optimal diet adherence (n=12)	100%			

*p-value calculated using t-test; p<0.05 test; p<0.001 Wilcoxon test
 †p-value significant at 5% level

► Multiple linear regression model adjusted for confounders of age, sex, BMI and regular physical activity showed a statistically significant change in eNO for the intervention group [95%CI: -27.35, -0.91; beta = -14.15; p=0.037] (Table 2). Specifically, one unit increase in fatty fish intake reduced bronchial inflammation by 54 ppb for children in the intervention group as compared to the control.

Table 2. Mean change in eNO

Variable	p	95% CI	p
Group	-0.45	-27.35, -0.91	0.04
Age	-1.69	-4.97, 1.59	0.31
Sex	6.89	-4.23, 21.33	0.20
Regular physical activity	1.69	-11.55, 14.96	0.80
BMI	0.22	-1.64, 2.09	0.81

*p = p-value calculated using Multiple linear regression model adjusting for confounders of age, sex, regular physical activity and BMI
 †p= unstandardized beta

Conclusion

The present study suggests that dietary Ω3 fatty acids consumed as fatty fish might have a protective effect on bronchial inflammation in asthmatic children. Future clinical studies are recommended to replicate and confirm our findings.

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e-posters


2. 11th European Nutrition & Dietetics Conference, Jun 29th - Jul 1st 2017, Madrid, Spain.

11th European Nutrition and Dietetics Conference
June 29 - July 1, 2017

Does Mediterranean dietary pattern enriched with fatty fish improve respiratory function in asthmatic children? A Randomized Controlled Trial

M. M. Papamichael¹ PhD; Ch. Katsardis²; B. Erbas² PhD; Professor C. Itsiopoulos¹ PhD

¹ La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
² La Trobe University, Department of Public Health, Australia
³ National & Kapodistrian University of Athens, Athens, Greece



Background	Materials & Methods	Application
Globally, asthma has rapidly become the most frequent allergic disease in children. It causes significant burden and is the most common reason for hospitalisation, absence from school and work for sufferers and their parents/careers, respectively [6], [7]. There is no cure for asthma, it can only be controlled by medication. Emerging evidence from observational studies indicate that diet and lifestyle play a role in the aetiology and management, with potential for a protective effect of a Mediterranean diet [1], [2]. In particular, fatty fish consumption has been associated with improved pulmonary function and reduced asthma symptoms in children [3], [4], [5]. However, randomized controlled trials are lacking.	Study Design: Parallel Randomized Controlled Trial Groups: a) Intervention group: Consumption of two fatty fish meals (≥150g cooked) per week as part of Greek Mediterranean Dietary pattern b) Control group: Consumption of usual diet Enrolment date: November 2016 Outcome measurements: baseline, 6 months Target population: Children aged 5-12 years old suffering from mild asthma Sample size: N=72 Recruitment: Paediatric Asthma Clinic, Greece Assessment Tools: → Pulmonary function: Spirometry (FEV1), Exhaled Nitric Oxide Analysis (eNO) → Asthma symptoms: Child Asthma Control Questionnaire (AACQ) → Quality of life: Paediatric Asthma Quality of Life (PAQLQ) Questionnaire → Dietary habits: Food Frequency Questionnaire (FFQ) → Adherence to Mediterranean Diet: KIDMED Index → Biomarkers: Plasma fatty acid composition, antioxidant levels, Vitamin D, Metabolic profile (Krebs cycle metabolites)	This study is important in establishing the effect of a Mediterranean diet enriched with fatty fish in the management of asthma in children. Findings will inform the development of dietary guidelines for asthma management in children. References [1] Arvaniti et al. 2011. <i>Pediatric Allergy Immunol</i> , 22(3), 203-209 [2] Garcia-Marcos et al. 2013. <i>Pediatric Allergy Immunol</i> 2013;00 [3] Hodge et al. 1995. <i>Medical Journal Australia</i> , 164(3), 137-140. [4] Kremenova et al. 2009. <i>Clin Rev Allergy Immunol</i> DOI: 10.1007/s12016-009-9166-6 [5] Magnusson et al. 2013. <i>Am J Clin Nutr</i> 2013;97:1324-30. [6] WHO, 2013. http://www.who.int/mediacentre/factsheets/fs307/en/ [7] GINA, 2017. http://ginasthma.org/2017

Aim
To investigate whether fatty fish consumption as part of a Greek Mediterranean dietary pattern improves pulmonary function and reduces asthma symptoms in children.

Clinical Trial Registration: ACTRN12616000492459p

Contact details: M. Papamichael
papapap@hotmail.com

3. 3rd International Conference on Respiratory & Pulmonary Medicine Jul 17-18, 2017, Melb., Australia

DOES A MEDITERRANEAN DIETARY PATTERN ENRICHED WITH FATTY FISH IMPROVE RESPIRATORY FUNCTION AND REDUCE ASTHMA SYMPTOMS IN CHILDREN?
A RANDOMIZED CONTROLLED TRIAL
REPORT OF BASELINE RESULTS

Maria M. Papamichael¹; Charis Katsardis²; Dimitris Tsoukalas³; Bircan Erbas²; Catherine Itsiopoulos¹

¹ La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
² La Trobe University, Department of Public Health, Australia
³ National & Kapodistrian University of Athens, Athens, Greece
⁴ European Institute of Nutritional Medicine, Rome, Italy




Background	Purpose	Methodology	Findings
Emerging evidence from observational studies indicates that diet and lifestyle play a role in the aetiology and management of chronic diseases such as obesity and asthma in children, with a potential for protective effect of a Mediterranean diet [1],[2],[3]. Previous studies have reported an association between overweight, development and severity of asthma in children [4],[5].	This is the first Randomized Controlled Trial (RCT) to investigate whether fatty fish consumption as part of a Mediterranean dietary pattern improves pulmonary function and reduces asthma symptoms in children.	Study design: RCT Duration: 6 months Participants: 72 Greek children (54% ♂, 46% ♀) with doctor-diagnosed 'mild asthma' Age: 5-12 years (Mean age: 8±2 y.o.) Recruitment: November 2016, paediatric asthma clinic in Athens, Greece Randomized equally into two groups: 1. Intervention group (n=36): instructed to consume 2 fatty fish meals per week (at least 150g cooked fish/meal) as part of the Greek Mediterranean diet. 2. Control group (n=36): their usual diet. Assessment tools: • Anthropometry: Weight, height, BMI • Pulmonary function: Spirometry (FEV1), eNO • Asthma Control: Asthma Control Questionnaire [6] • Quality of life: Paediatric Asthma Quality of Life Questionnaire [7] • Dietary habits: Food Frequency Questionnaire [8] • Adherence to Mediterranean diet: KIDMED score [9] • Assessment time-points: baseline & 6 months	Data analysis of baseline measurements reveals that 64% of children are 'normal' height and 36% 'tall'. Regarding bodyweight, 1% of children are 'severely underweight', 3% 'slightly underweight', 57% 'normal' weight, 28% 'overweight' and 11% 'obese' according to the Hellenic paediatric growth charts (Fig.1)



Fig 1. Figure 1 illustrates Children's weight distribution per gender. This diagram shows that girls are more overweight than boys (36.4% v 20.5%) and boys are more obese than girls (15.4% v 6.1%) respectively.

Conclusion
This observation is important since BMI seems to be a major risk factor in paediatric asthma. Future public health strategies should focus on promoting a healthy diet similar to the Mediterranean diet, daily physical activity and maintenance of a healthy weight in the management of childhood asthma.

References: [1] Arvaniti et al. 2011. *Pediatric Allergy Immunol*, 22: 203-209.
[2] Garcia-Marcos et al. 2013. *Pediatric Allergy Immunol*, 24: 100-104.
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RCT No.: ACTRN12616000492459p

Contact details:
M. Papamichael
papapap@hotmail.com

4. 14th International Conference on Clinical Nutrition, July 27-29 2017, Rome, Italy.

A Mediterranean diet enriched with ω 3-polyunsaturated fatty acids in the management of paediatric asthma. A Randomised Control Trial

M.M. Papamichael¹ PhD; Ch. Katsardis² MD PhD; D. Tsoukalas⁴ MD; B.Erbas² PhD; C. Itsiopoulos¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Melbourne, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

Background

Asthma is an inflammatory disease in the lungs which over the past thirty years has escalated in children [1], [2]. Considerable interest exists in the therapeutic potential of dietary omega 3 fatty acids, due to anti-inflammatory and immune-modulating effects on asthma [3],[4],[6]. However, studies performed to date are inconclusive and this requires further exploration [5], [7], [8].

Aim

This six month Randomized Controlled Trial aims to investigate whether fatty fish as part of the Greek Mediterranean diet reduces asthma symptoms in children.

Clinical Trial No. : ACTRN12616000492459p

Materials & Methods

► Study Design: Parallel Randomized Controlled Trial

► Groups:

a) **Intervention group:** Consumption of two fatty fish meals (≥ 150 gr cooked) per week as part of Greek Mediterranean Dietary pattern

b) **Control group:** Consumption of usual diet.

► Enrolment date: November 2016

► Target population: Children aged 5-12 years old suffering from mild asthma

► Sample size: n=64

► Recruitment: Paediatric Asthma Clinic, Greece

► Outcome measurements: baseline, 6 months

► Assessment Tools:

1. Pulmonary function: Spirometry (FEV1), Exhaled Nitric Oxide Analysis (eNO).
2. Asthma symptoms: Child Asthma Control Questionnaire (ACQ)
3. Quality of life: Paediatric Asthma Quality of Life (PAQLQ) Questionnaire
4. Dietary habits: Food Frequency Questionnaire (FFQ)
5. Adherence to Mediterranean Diet: KIDMED Index
6. Biomarkers: Plasma fatty acid composition, antioxidant levels, Vitamin D, Metabolic profile (Krebs cycle metabolites)

Application

This study intends to establish whether fatty fish consumption can be used as an adjunct therapy in the management of asthma in children.

References

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Contact details: M. Papamichael
saspap@icloud.com

8. 17th Global Dieticians & Nutritionists Annual Meeting October 2-3, 2017 Kuala Lumpur, Malaysia

A Mediterranean diet enriched with ω 3-polyunsaturated fatty acids in the management of paediatric asthma. A Randomised Controlled Trial: Baseline results

M.M. Papamichael¹ PhD; Ch. Katsardis² MD PhD; D. Tsoukalas⁴ MD; B.Erbas² PhD; C. Itsiopoulos¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Melbourne, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

Background

The change in dietary patterns has contributed to the rise in obesity and asthma in children [1],[2]. Both chronic diseases are associated with co-morbidities, considerable disability, poor quality of life and increase in medical costs [3]. Research studies have demonstrated that, an elevated BMI is related to an increase in asthma risk and development of future exacerbations, less asthma control and an increase need for medication use [4],[5],[6].

Aim

The purpose of this Randomized Controlled Trial is to investigate the effect of a Mediterranean diet enriched with fatty fish on asthma in Greek children. This is the first announcement of baseline results.

Materials & Methods

Study Design: Parallel Randomized Controlled Trial

- Participants: 72 children (34.2% ♂, 45.8% ♀)
- Ages: 5-12 y.o with doctor-diagnosed 'mild-asthma'
- Randomized into 2 groups:
- a) **Intervention group (n=36):** Consumption of two fatty fish meals/week (≥ 150 gr cooked per meal) as part of Greek Mediterranean Dietary pattern over a period of 6 m.o
- b) **Control group (n=36):** Consumption of usual diet.

Assessment Tools:

- Pulmonary function: Spirometry (FEV1), eNO
- Asthma Control: Asthma Control Questionnaire [7]
- Quality of life: Paediatric Asthma Quality of Life Questionnaire [8]
- Dietary habits: FFQ [9]
- Adherence to Mediterranean diet : KIDMED test [10]

Statistical Analysis

SPSS IBM (Version 20) was used for all statistical compilations. Continuous variables were checked for normality using numerical and graphical methods. Differences between intervention groups were assessed using t-test for variables found to be normally distributed and Mann-Whitney for non-normal variables. P-value was considered to be statistically significant at the 5% level.

Results

Statistical analysis of baseline data reveals that 64% of children are 'normal' height and 36% are 'tall'. Regarding bodyweight, 1% of children are 'severely underweight', 3% 'slightly underweight', 57% 'normal' weight, 28% 'overweight' and 11% 'obese' according to Hellenic paediatric growth charts.

Conclusion

This finding is significant since BMI seems to play a major role on asthma outcome in children. The effect of weight reduction in overweight asthmatic children might be of great value for current treatment guidelines and in alleviation of asthma symptoms.

References

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Contact details: M. Papamichael
saspap@icloud.com

18. 28th International Conference on Pediatrics Health, Aug 12-13, 2019, Rome, Italy

28th International Conference on
Pediatrics Health
August 12-13, 2019 Rome, Italy

Urinary metabolomic profile of Greek asthmatic school-children

M.M. Papamichael¹; Ch. Katsardis²; D. Tsoukalas³; B. Erbas⁴; C. Itsiopoulou^{1,5}

¹ La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Nutrition & Sport, Australia.
² National & Kapodistrian University of Athens, Athens, Greece
³ European Institute of Nutritional Medicine, Rome, Italy
⁴ La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia
⁵ Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia.

Introduction

In clinical practice, biomarkers can provide complementary information to conventional pulmonary tests, symptoms, spirometry, exhaled nitric oxide, PC20methacholine and histamine⁽¹⁾. They are useful in establishing diagnosis, monitoring of the disease progression and in response to treatment. The development of non-invasive sampling methods and detection techniques for the identification of components involved in airway inflammation including the determination of biomarkers, would greatly contribute to our current insight in airway inflammation associated with various asthma phenotypes as well as to customize individually-targeted therapies. In children, reliable, non-invasive biomarkers would be valuable ⁽¹⁾. We applied metabolomic analysis to study the association between urinary organic acid concentrations and pulmonary function in 72 Caucasian children (5-12 years) with asthma determined by gas chromatography and mass spectrometry (GC-MS).

Methods

Study design: Dietary intervention study

Recruited: 72 children (54% Male; Mean age 8 ± 2 y.o) from Nov 1st-Dec 31st, 2016.

Venue: Pediatric asthma clinic, Athens, Greece

Inclusion criteria: Doctor-diagnosed 'mild asthma' ⁽²⁾

Pulmonary function: Spirometry (FEV₁, FVC, FEV₁/FVC, PEF, FEF_{25-75%}) ⁽³⁾

Bronchial inflammation: exhaled Nitric Oxide (eNO) ⁽⁴⁾

Asthma control: Asthma Control Questionnaire ⁽⁵⁾

Urinary Organic Acid (OA) Profile: GC-MS ⁽⁶⁾

Time-point: Baseline

Results

- 34 unique urinary organic acids identified by targeted metabolomics (Fig.1)
- 8 significant correlations found between urinary OA and spirometry, eNO and ACQ scores (p< 0.05) (Table 1)
- Difference in lactic acid concentration between boys and girls (5.82 ± 6.48 vs 6.27 ± 3.45 mmol/mol Crea; p=0.03)

Fig 1. Mean Concentration of 34 urinary OA of asthmatic children

Table 1. Correlation between OA and pulmonary function tests and asthma control

OA vs Pulmonary function	P	Spearman's rho
Lactic vs FVC	0.03	-0.27
Lactic vs FEV ₁	0.02	-0.29
4-hydroxyphenylacetic vs FVC	0.01	-0.32
4-hydroxyphenylacetic vs FEV ₁	0.01	-0.32
5-hydroxyindoleacetic vs FEV ₁ FVC	0.03	0.28
Glycolic vs PEF	0.03	-0.27
5-hydroxyindoleacetic vs eNO	0.05	-0.24
Malic vs ACQ score	0.01	-0.64
Glutamic vs ACQ score	0.05	-0.95
2-hydroxyisobutyric vs ACQ score	0.02	0.29

Conclusion: Biomarkers such as urinary organic acid can be useful tools in clinical practice for the diagnosis and management of asthma in children as well as in proposing novel therapeutic targets.

Contact: Maria. M. Papamichael
E-mail: sassipap@hotmail.com

References

1. Meschino et al, 2015. *Pediatr Respir Rev* 16: 205-212
2. GINA, 2016. <http://ginasthma.org/wp-content/uploads/2016/04/GINA-Appendix-2016-final.pdf>
3. Miller et al, 2005. *Eur Respir J* 26:519-38.
4. Dweik et al, 2011. *Am J Respir Crit Care Med* (184): 602-615
5. Juniper et al, 2006. *Respir Med* 100:616-21.
6. Tanaka et al, 1990. *Clin Chemistry*, 26:1839-46

Video presentations

5. 10th American Pediatric Healthcare & Pediatric Infectious Diseases Congress, Sep 20-22 Toronto, 2017 Canada.

10th American Paediatrics HealthCare Congress
September 20-22, 2017; Toronto, Canada

Can dietary Omega 3 fatty acids reduce asthma symptoms in children?
Preliminary results of a Randomized Controlled Trial

M.M. Papamichael¹ PhD; Ch. Katsardis² MD PhD; D.Tsoukalas⁴ MD; B.Erbas³ PhD; C.Itsiopoulos¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy



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 - Prevalence
 - Asthma management
2. Mediterranean Diet Therapy
3. Randomized Controlled Trial
 - Methods
 - Study design
4. Baseline results
5. Next steps
6. Conclusion
7. References



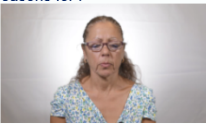
What we know

The rise in paediatric asthma prevalence has become a **major public health concern globally**. It is one of the most common reasons for :

- ✓ Hospitalization
- ✓ Days absent from school
- ✓ Parents time off work
- ✓ Limits every day activities
- ✓ Affects sleep patterns and concentration
- ✓ Decreases quality of life (QOL) for the child and family
- ✓ Causes economic burden due to an increase in medical costs

It has been estimated that asthma accounts for a loss of 10 million school days annually, among children and adolescents, 5-17 y.o.


Ref: WHO, 2017. Asthma. Retrieved from <http://www.who.int/mediacentre/factsheets/fs307/en/>
European Respiratory Society, 2015. Retrieved from www.erswhitebook.org
Global Initiative for Asthma (GINA), 2016. Retrieved from <http://www.ginasthma.org>



What we know

Research has shown that the asthma epidemic in children is attributed to **genetic and environmental factors** including **lifestyle and diet**. It has been postulated that the modern diet which consists of a high intake of fast food, margarine and vegetable oils, food sources **high in Omega 6 fatty acids** and a low intake of fish, which is rich in **Omega 3 fatty acids** is responsible for the rise in asthma prevalence in children (Seaton, 1994 Black & Sharpe, 1997)

Seaton et al, 1994. Thorax; 49: 171-174
Black & Sharpe, 1997. Eur Respir J, 1997; 10: 6-12




International Study of Asthma & Allergy in Childhood (ISAAC)

World map of prevalence of asthma in children

↑ English-speaking (UK, Aust, US, NZ)
↓ Mediterranean (Gr, It, Sp, Al)

5-7.5%	2.5-5%
0-2.5%	7.6-10%
>10%	No data

Ref: Asher, 2006 Lancet 368: 723-43
Beasley, et al, 1998 Lancet, 351: 1125-1132



ASTHMA MANAGEMENT

- There is **NO CURE** for asthma.
- Symptoms can only be controlled by **medication** → side-effects?




Mediterranean diet therapy?

What about diet ???




Mediterranean diet therapy?

Numerous studies examining the effect of individual nutrients or food groups on asthma have reported inconsistent results and failed to show a causal relationship. For this reason there is growing interest on the impact of dietary patterns in chronic disease.




7.15th World Congress on Advances in Nutrition, Food Science & Technology, September 11-12, 2017 Edinburgh, Scotland

15th World Congress on Advances in Nutrition, Food Science & Technology; Edinburgh, Scotland, September 11-12, 2017

GREEK CHILDREN SUFFERING FROM ASTHMA ABANDON MEDITERRANEAN DIETARY PATTERN. BASELINE RESULTS

M.M. Papamichael¹ PhD; Ch. Katsardis² MD PhD; D.Tsoukalas⁴ MD; B.Erbas² PhD; C.Itsiopoulos¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

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Paediatric Asthma

WHAT WE KNOW

Asthma is the most **common chronic** disease in **children** that has **risen globally** over the past two to three decades and has become a **major public health concern**.

It is associated with an increase in morbidity, mortality and burden for the individual, family and society.

Ref: European Respiratory Society, 2015.
GINA, 2014.
WHO, 2017.

WHAT WE KNOW

Childhood asthma is one of the most common reasons for :

- ✓ Hospitalization
- ✓ Days absent from school
- ✓ Parents time off work
- ✓ Limits every day activities
- ✓ Decreases quality of life (QOL) for the child and family
- ✓ Causes an economic burden due to an increase in medical costs

It has been estimated that asthma accounts for a loss of 10 million school days annually, among children and adolescents, 5-17 y.o. (GINA, 2014).

Ref: WHO, 2017. Asthma <http://www.who.int/mediacentre/factsheets/fs307/en/>
European Respiratory Society, 2015.
Global Initiative for Asthma (GINA), 2014

MEDITERRANEAN DIET THERAPY?

Numerous studies examining the effect of individual nutrients or food groups on asthma have reported inconsistent results and failed to show a causal relationship. For this reason there is growing interest on the impact of dietary patterns in chronic disease.

MEDITERRANEAN DIET THERAPY?

Front Pediatr. 2017 Apr 21;5:72. doi: 10.3389/fped.2017.00072. eCollection 2017.

What Are the Effects of a Mediterranean Diet on Allergies and Asthma in Children?

Castro-Rodriguez JA¹, Garcia-Marcos L².

The systematic literature search identified 15 studies, of which 7 are recent and 8 were included in an earlier meta-analysis undertaken in 2013. Regarding the 7 recent studies, 4/7 reported that high adherence to the Mediterranean diet was 'inversely' related to asthma symptoms, 2/7 'no effect' and 1/7 studies an 'increase' in asthma prevalence in girls.

Overall, it was concluded that, **Mediterranean diet** has a '**protective effect**' on asthma in children.

MEDITERRANEAN DIET THERAPY?

What about fish?

There is growing interest in the use of Omega 3 fatty acids for inflammatory diseases including asthma due to anti-inflammatory and immuno-modulating effects.

Some studies have reported that fish consumption resulted in an improvement in pulmonary function and lower asthma symptoms in children but '**clinical trials are lacking!**'.

Ref: Kull et al. 2006. Allergy, 61(8), 1009-1015
Hodge et al. 1996. The Medical journal of Australia, 164(3), 137-140
Magnusson et al. 2013. The American journal of clinical nutrition, 97(6), 1324-1330
Calder, 2013. Br J Clin Pharmacol, 75(2): 645-662

The prophylactic potential of a Mediterranean dietary pattern enriched with fatty fish in asthmatic children: A Randomized Controlled Trial


Protocol No: ACTRN12616000492459p

Purpose:

To investigate whether an increase in fatty fish consumption in the context of a Mediterranean diet reduces asthma symptoms in Greek children.

12. World Congress on Nutrition & Dietetics, June 18-19 2018, Paris France

World Congress on
NUTRITION & DIETETICS, June 18-19, 2018 Paris, France
Theme : Promulgating latest Innovations and Applications in the field of Nutrition & Dietetics



World Nutrition 2018

Fatty fish (Ω3) - A diet therapy for paediatric asthma?



Papamichael Maria Michelle¹ PhD; Katsardis Charis³ PhD; Koutsilieris Michael³ PhD; Tsoukalas Dimitris⁴ MD; Lambert Katrina² PhD; Erbas Bircan² PhD; Itsiopoulos Catherine¹ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.

²La Trobe University, Department of Public Health, Melbourne, Australia

³National & Kapodistrian University of Athens, Athens, Greece

⁴European Institute of Nutritional Medicine, Rome, Italy



Overview

1. Background
 - Epidemiology
 - Pathophysiology
 - Diet and asthma
2. RCT
 - Methods/Study Protocol
3. Results
 - Baseline
 - Six months
4. Conclusion



Asthma Epidemic

300 million people currently suffer from asthma¹

It is estimated that by 2025, there will be an additional 100 million people².



REF: 1. World Health Organization (WHO), 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>
2. WHO, 2007. <http://www.who.int/gard/publications/GARDi20Book3202007.pdf>



Asthma can kill

In 2015, asthma accounted for **383,000 deaths (WHO)**^{1,2}



REF: 1. WHO, 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>
2. Global Initiative for Asthma (GINA), 2016. <http://www.ginasthma.com/>

International Study of Asthma & Allergy in Childhood (ISAAC)

World map of prevalence of asthma in children

Hi: English-speaking¹

(UK, Aust, US, NZ)

Lo: Mediterranean², Middle East³, Asia²



REF: 1. Asher, 2006. Lancet 2006; 368: 733-43
2. Beasley, et al, 1998. The Lancet, vol. 351, pp. 1125-1132
3. Mirzaei, 2017. Medical Journal of the Islamic Republic of Iran, 31, 9.



Childhood Asthma-Public Health Concern

- Asthma is the most common chronic disease in children that has risen globally over the past 30 years
- It starts early in life and may continue into adulthood¹
- 50-80% of children develop asthma by 5 years old
- It is more prevalent in boys < 10 years
- girls > 10 years of age^{1,2}



REF: 1. Global Initiative for Asthma (GINA), 2016. <http://www.ginasthma.com/>
2. WHO, 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>



Childhood Asthma- Burden

Most common reasons:

- Hospitalization^{1,2}
- Days absent from school^{1,2}
- Parent's time off work³



Asthma accounts for a loss of 10 million school days annually, among children and adolescents, 5-17 y.o.²

REF: 1. GINA, 2016. <http://www.ginasthma.com/>
2. WHO, 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>
3. Tsakiris et al, 2013. BioMed Research International, 2013: 1-7.



13. 17th American Pediatrics Healthcare & Infectious Diseases Congress June 27-28th 2018, Vancouver, Canada

17th American Pediatrics Healthcare & Infectious Diseases Congress
June 27-28 2018, Vancouver, Canada

The prophylactic potential of fatty fish consumption on airway inflammation in childhood asthma

Papamichael Maria¹ RD; Katsardis Charis³ MD; Koutsilieris Michael³ MD; Tsoukalas Dimitris⁴ MD; Lambert Katrina² STAT; Erbas Bircan² STAT; Iliopoulos Catherine¹ APD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Australia.
²La Trobe University, Department of Public Health, Australia
³National & Kapodistrian University of Athens, Greece
⁴European Institute of Nutritional Medicine, Italy



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Content

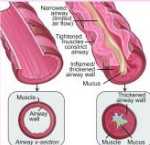
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 - Epidemiology
 - Pathogenesis
 - Etiology
2. *Evidence-based medicine*
 - Omega 3/6 fatty acids
 - Fish intake & pediatric asthma: A meta-analysis
3. *Clinical trial*
 - Methods/Study design
 - Results
4. *Conclusion*




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Definition

- Asthma is a chronic respiratory disorder in the lungs in which inflammation causes bronchi to swell, muscle constriction, mucus production, that lead to airway obstruction and hyperresponsiveness^{1,2}

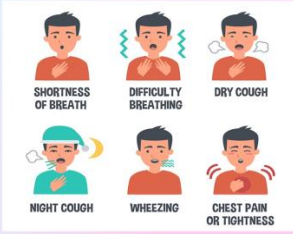


Ref: 1. Papadopoulos et al, 2012. *Allergy* 67 (2012) 976–997
2. Doehring, 2013. *J Appl Physiol* (1985) 2013 Apr 1; 114(7): 834–843



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Symptoms





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Common disease in children-public health concern!

- Asthma is the most common allergic disease in children that continues to rise globally¹
- In U.S 6.2 million children under 18 years suffer with asthma²
- It starts early in life, may persist into adulthood^{1,3}
- More common in boys <10 y.o⁴ in girls > 10 y.o





Ref: 1. Fuchs et al, 2017. *Respiratory Medicine*, May, 5(3):224-234
2. CDC Statistics, 2015. National Health Interview Survey, 2015 <https://www.cdc.gov/nchs/fastats/asthma.htm>
3. Asher, 2014. *INT J TUBERC LUNG DIS* 18(11):1209–1218
4. GINA, 2016. Available at ginasthma.org




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Pediatric asthma- decreases quality of life

- It creates substantial burden to individuals & families due to need for hospital admissions and emergency medical care
- Recurrent attacks cause sleeplessness, tiredness, lack of concentration, poor academic performance & school absenteeism
- Restricts daily activities
- Impacts the quality of life for the patient and family^{1,2}


Ref: 1. WHO, 2017. Asthma Fact sheet. Available from <http://www.who.int/mediacentre/factsheets/fs302/en/>
2. Nunes et al. *Asthma Research and Practice* (2017) 3:1



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Financial burden for individual and society

- ↑ Financial burden for individual and society due to need for medical care, asthma medication and parents time off work¹
- It has been estimated that the mean cost of asthma is \$ 3100 USD per patient annually in the US and \$1900 USD in Europe¹
- According to U.S statistics, it costed caretakers \$726.1 million per year because of work absence due to asthma².





Ref: 1. Nunes et al, 2017. *Asthma Research and Practice* 3:1 DOI 10.1186/s40733-016-0029-3
2. Sharma et al, 2014. Available from <http://emmedicine.medicape.com/article/1000997overview#5>


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Asthma is underdiagnosed and undertreated!

- The goal of asthma management is prevention of symptoms and risk of future exacerbations as well as to lead a normal, active life^{1,2}.
- Medication is the cornerstone of asthma treatment^{1,2}

Ref: 1. GINA, 2016. Available at www.ginasthma.org
2. Papadopoulos, 2012. *Allergy* 67 (2012) 976–997



14.14th International Congress on Advances in Natural Medicines, Nutraceuticals & Neurocognition, July 19-20, 2018, London, UK



14th International Conference on Advances in Natural Medicines, Nutraceuticals & Neurocognition, July 19-20, London, UK

Omega 3 fatty acids- A new therapeutic target for childhood asthma?

Papamichael Maria, M PhD¹; Katsardis Charis MD PhD²; Koutsilieris Michael MD PhD³; Tsoukalas Dimitris MD⁴; Lambert Katrina PhD²; Erbas Bircan PhD²; Itsiopoulos Catherine PhD¹

¹ La Trobe University, Department of Rehabilitation, Nutrition & Sport, Australia.
² La Trobe University, Department of Public Health, Australia
³ National & Kapodistrian University of Athens, Greece
⁴ European Institute of Nutritional Medicine, Italy

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1. Background
 - 1.1 Paediatric Asthma
 - 1.2 Asthma burden
 - 1.3 Cost of asthma
 - 1.4 Prevalence
 - 1.5 UK Statistics
 - 1.6 Asthma control/management
 - 1.7 Aetiology
 - 1.8 Asthma exacerbations-symptoms
2. Diet theories-Eskimos
3. Omega 3 fatty acids
 - 3.1 Mode of action
4. Evidence from Meta-analysis
5. Randomized Controlled Trial
 - 5.1 Methods/Study design
 - 5.2 Results
6. Conclusion

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PAEDIATRIC ASTHMA-PUBLIC HEALTH CONCERN

- The rise in paediatric asthma prevalence has become a major public health concern globally^{1,2}.
- Most common reason for:
 - Hospitalization, emergency visits for medical care
 - Days absent from school
 - Affects sleep patterns, concentration, academic performance
- It has been estimated that asthma accounts for a loss of 13 million school days annually, among children and adolescents, 5-17 y.o.^{2,3}

1. WHO, 2017. Asthma. Available from <http://www.who.int/mediacentre/factsheets/fs307/en/>
 2. Global Initiative for Asthma (GINA), 2016. Available from www.ginasthma.org/
 3. Akilovs L., 2010. Pediatric Allergy, Immunology, and Pulmonology, 2010; DOI: 10.1089/jped.2010.0013

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ASTHMA BURDEN

- Affects the child socially, physically and psychologically
- Limits daily activities
- Decreases Quality of Life (QOL) for the child and family
- Parents time off work

Ref:
 1. WHO, 2017. Asthma. Available from <http://www.who.int/mediacentre/factsheets/fs307/en/>
 2. Global Initiative for Asthma (GINA), 2016. Available from www.ginasthma.org/
 3. Sharma et al., 2014. Available from <http://www.medicines.medscape.com/article/1000997/overview/a5>

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UK STATISTICS^{1,2}

- The UK has one of the highest rates of asthma in children worldwide
- According to NHS, asthma is one of the most common long term conditions affecting 1.1 million children (1 in 11).
- Approx. 75% of hospital admissions are for asthma
- > 70 children are admitted to hospital daily due to asthma
- Every 20 minutes a child is admitted because of an asthma attack

Ref: 1. NHS, 2018. Asthma: Better for less. Available from <https://www.networks.nhs.uk/docs/networks/respiratory-leads/yorkshire-leads/yorkshire-leads-respiratory-programme/documents/Better%20for%20less%20-%20%20%20>
 2. Asthma (UK), 2016. <https://www.asthma.org.uk/about/media/facts-and-statistics/>

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ASTHMA COSTS

- Economic burden due to an increase in medical costs^{1,2}
- It has been estimated that asthma costs the NHS at least £1.1 billion a year, for hospital admissions, medication, and over £3.7 million for GP visits that are preventable by treatment².

Ref: 1. Asthma UK, 2014. <https://www.asthma.org.uk/about/networks/communications/communications-press-centre/2014.pdf>
 2. NHS, 2018. Asthma: Better for less. Available from <https://www.networks.nhs.uk/docs/networks/respiratory-leads/yorkshire-leads/yorkshire-leads-respiratory-programme/documents/Better%20for%20less%20-%20%20%20>

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ASTHMA CONTROL IS POOR

- Asthma control is poor in children, as a consequence of non-compliance with therapy^{1,2}.
- In 5% of children, have chronic symptoms and recurrent episodes despite maximum treatment with conventional medications¹.

***Hence, there is a need for a therapeutic tool that is effective in improving control and reducing asthma burden in children.**

Ref: 1. Hadlin et al., 2012. Eur Respir Rev 21: 175-185
 2. NHS, 2018. Asthma: Better for less. Available from <https://www.networks.nhs.uk/docs/networks/respiratory-leads/yorkshire-leads/yorkshire-leads-respiratory-programme/documents/Better%20for%20less%20-%20%20%20>

15 3rd World Congress on Nutrition, Dietetics and Nutraceuticals, Feb 25th 2019, Prague, Czechslovakia



The importance of vitamin D status on lung function in asthmatic children

Papamichael M. M¹; Katsardis Ch.³; Koutsilieris M³; Tsoukalas D.⁴; Lambert K.²; Erbas B. ²; Itsiopoulos C.¹

¹La Trobe University, School of Allied Health, Human Services & Sport, Department of Human Nutrition & Sport, Melbourne, Australia.
²La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

OVERVIEW

1. What we know about
 - Asthma
 - Vitamin D
 - Evidence on Vitamin D deficiency and childhood asthma
2. Methods of RCT
3. Results
4. Conclusion

Asthma

- ~ 300 million people currently suffer from asthma ¹
- By 2025 there will be an additional 100 million ²

1. World Health Organization (WHO). 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>
 2. WHO. 2007. <http://www.who.int/gard/publications/GARD%20Book%202007.pdf>

Vitamin D status in children/adolescents (1-18 years worldwide)^{1,2}

Low 25 (OH)D < 50 nmol/L	High 25 (OH)D > 50 nmol/L
Greece	North America
Italy	Sweden
Spain	
Germany	
Finland	
Austria	
South America	
Mongolia	

*where < 50 nmol/L insufficient (IOM, 2011)

1. International Osteoporosis Association & DSM Nutritional Products. 2012. http://revista-fi.com.br/upload_arquivos/201606/2016060608176001464973585.pdf
2. Manios. 2018. Eur J Nutr. 2018 Sep;57(6):2001-2036.

Vitamin D deficiency & childhood asthma

Vitamin D deficiency in asthmatic children is related to :

- ✓ ↑ Asthma prevalence ^{1,2,3}, exacerbations ^{4,5,6}
- ✓ ↑ Hospitalization & emergency visits ^{7,8}
- ✓ ↑ Upper respiratory infections ^{9,12}, medication use ^{5,6,11}
- ✓ ↑ Airway hyperresponsiveness ^{9,10}, airway remodeling ⁶
- ✓ ↓ Poor lung function (FEV₁, FVC, FEV₁/FVC)^{1,4,6}, poor asthma control ^{6,7,13,14}

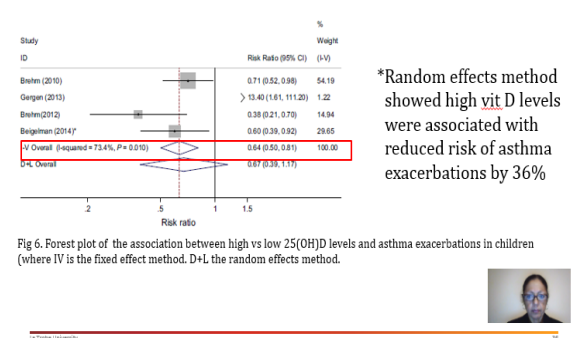
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2. Bener. 2012. Int Arch Allergy Immunol. 157(2):168-75.
3. Nirubans. 2011. Allergy Asthma Immunol Res. 3(4):251-255.
4. Brehm. 2012. Am J Respir Crit Care Med. 186(2):140-146.
5. Dogru. 2014. Int Arch Allergy Immunol. 164(4):319-25.
6. Gupta. 2011. Am J Respir Crit Care Med. 184(12):1342-1349.
7. Uysalol. 2013. Italian J Pediatr. 39:78.
8. Brehm. 2010. J Allergy Clin Immunol. 126:52-8.
9. Brehm. 2009. Am J Respir Crit Care Med. 179:765-771.
10. Hollams. 2011. Eur Respir J. 38: 1320-1327.
11. Searring. 2010. J Allergy Clin Immunol. 125(5):995-1000.
12. Science. 2013. Clinical Infectious Diseases 57(3):392-7.
13. Chinnellato. 2011. J Pediatr. 158:457-41.
14. Kaaviyaa. 2018. Indian Pediatr. 55(11):969-971.

Allergy. 2015 Apr;70(4):339-54. doi: 10.1111/all.12583.

The role of circulating 25 hydroxyvitamin D in asthma: a systematic review.

Cassim R¹, Russell MA, Lodge CJ, Lowe AJ, Koolin JJ, Dharmage SC.

- A systematic review & meta-analysis of observational studies that included both children and adult patients
 - 340 publications, 23 relevant studies (12 cohort, 9 cross-sectional, and 2 case-control)
 - 18 studies were conducted on children/adolescents, 5 on adults
 - In summary, higher vitamin D levels were associated with decreased risk of acute asthma exacerbations, although there was little evidence to suggest an association between vitamin D levels with prevalence, incidence, and severity of asthma, most likely due to high heterogeneity among study methodologies.
-



16. 20th International Congress on Nutrition and Health, Mar 28-30, 2019, Stockholm, Sweden.



Contents

- Vitamin D status worldwide
- Sources of vitamin D
- Vitamin D metabolism
- Vitamin D and respiratory health
- Vitamin D deficiency & asthma: The evidence
- Pediatric asthma
- Details of RCT:
 - Methodology
 - Results
 - Main Findings
- Conclusion



La Trobe University

The synergistic effect of vitamin D in pediatric asthma



Papamichael M. M.¹; Lambert K.²; Tsoukalas D.⁴; Koutsilieris M.³; Katsardis Ch.³; Erbas B. ²; Itsiopoulos C.¹

¹ La Trobe University, School of Allied Health, Human Services & Sport, Department of Dietetics, Human Nutrition & Sport, Melbourne, Australia.

² La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne, Australia

³ National & Kapodistrian University of Athens, Athens, Greece

⁴ European Institute of Nutritional Medicine, Rome, Italy



La Trobe University

2



Vitamin D deficiency pandemic!

- In the last few decades the worldwide prevalence of hypovitaminosis D in children and adults has emerged as a major public health issue even in regions with abundant sunshine^{1,2}
- At least 1 billion people are deficient in vitamin D due to limited sun exposure, sun-screen use, living in higher latitudes, season, dark skin pigmentation, coverage with clothing and inadequate dietary intake^{1,2}.



1. Holick 2008. BMJ 2008; 336 doi: <https://doi.org/10.1136/bmj.39581.411424.80>

2. Cashman. 2016. Am J Clin Nutr 2016;103:1033-44

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Eur J Nutr. 2018 Sep;57(6):2001-2036. doi: 10.1007/s00394-017-1564-2. Epub 2017 Oct 31.

A systematic review of vitamin D status in southern European countries.

Mariño Y¹, Moschonis G², Lambriou CP³, Tsouloukouliou K⁴, Rinau P⁵, Karachaliou A⁶, Bresidenassel G⁴, Gonzalez-Gross M⁴, Kivimäki M⁷, Cashman M^{8,9}.

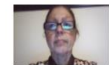
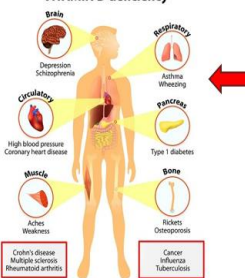
- Analysed 107 studies, 630,093 children and adults were assessed
- >33% of studies reported mean 25(OH)D < 50 nmol/L (20 ng/ml)
- ~ 10% studies mean 25(OH)D < 25 nmol/L
- 40.4% of European population have low serum 25(OH)D < 50nmol/L
- High prevalence of low vitamin D status in Southern Europe and Eastern Mediterranean regions, despite abundant sunshine: Italy, Spain, Turkey, France, Israel, Greece, Cyprus, Portugal



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Consequences of vitamin D deficiency on health

VITAMIN D deficiency

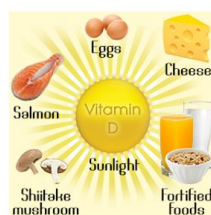


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7



Sources of Vitamin D¹



Dietary supplements

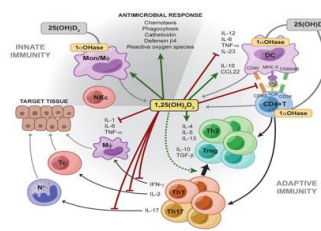


1. Buttriss. 2015. Nutrition Bulletin, 40, 279-28

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5

Vitamin D- a potent modulator of inflammatory response¹



Vitamin D causes:

- Shift from Th1 to Th2 phenotype
- Inhibition of Th1, Th9, Th17
- ↓ pro-inflammatory cytokines (IL17, IL13)
- ↑ Promotes Th2, Treg
- ↑ anti-inflammatory cytokine (IL4, IL5, IL10)
- ↑ Phagocytic activity
- ↑ Enhanced steroid response
- ↑ Cathelicidin (anti-microbial peptide)
- ↓ production Ig E from B cell
- ↓ smooth muscle remodelling



1. Baekke. 2010. Pediatr Nephrol.25(9):1597-606.

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13

19. 2nd World Congress on COPD, Asthma and Lung Health, October 9-10, 2019, Madrid, Spain



Overweight/obesity increases ventilatory capacity and reduces FeNO in asthmatic children

Maria M Papamichael¹; Charis Katsardis³; Dimitris Tsoukalas⁴; Bircan Erbas²; Catherine Itsiopoulos^{1,5}

¹La Trobe University, School of Allied Health, Human Services & Sport, Dept. of Dietetics, Human Nutrition & Sport, Melbourne, Australia.

²La Trobe University, School of Psychology and Public Health, Dept. of Public Health, Melbourne, Australia

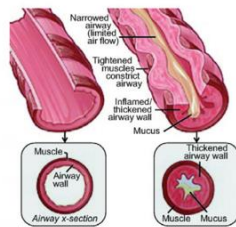
³National & Kapodistrian University of Athens, Athens, Greece

⁴European Institute of Nutritional Medicine, Rome, Italy

⁵Murdoch University, College of Science, Health, Engineering and Education, Perth, Australia.



Asthma is a chronic inflammatory disorder in the airways¹



- Airway inflammation
- Narrowing of airways
- Mucus production
- Hyper-responsiveness¹

Fig 1. Schematic representation of airway inflammation associated with asthma

1. World Health Organization (WHO), 2018. <http://www.who.int/mediacentre/factsheets/fs307/en/>

2. Deering, 2013. *J Appl Physiology* Apr;114(7):834-43



Lifetime prevalence of pediatric asthma across Europe¹

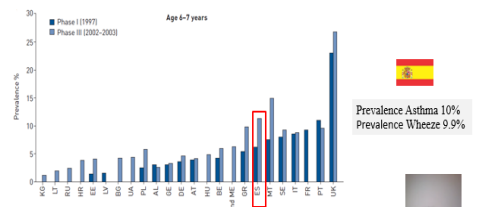


Fig 3. Lifetime prevalence of childhood asthma in Europe

1. European Respiratory Society, 2012. https://www.erswhitebook.org/chapters/11_childhood_asthma

2. Carvajal-Urueña, et al 2005. *Arch Bronconeumol*. 41(12):659-66



398,000 European children (6-9 years) Severely Obese!¹

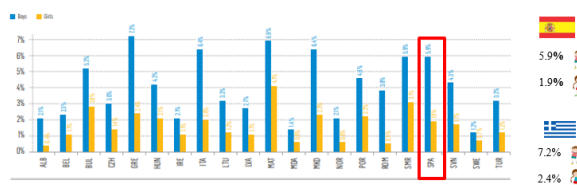


Fig 5. Prevalence of Severe Obesity among boys and girls 6-9 years (2007-2013)

1. WHO, 2013. <http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/activities/who-european-childhood-obesity-surveillance-initiative-cost>



Childhood Obesity medical complications

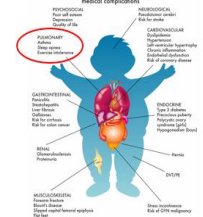


Fig. 1. Child obesity health related complications

1. Sahoo et al 2015. *J Family Med Prim Care*. Apr-Jun; 4(2): 187-192

2. Bellu, 2004. *J Clinical Endocrinol & Metab*. 89(6): 2587-2593. <https://doi.org/10.1210/yc.2004-0535>



Effect of overweight/obesity vs normal weight asthmatic children

- ↑Daily asthma symptoms¹
- ↑Days hospitalized¹
- ↑Missed school days¹
- ↑Increased use of reliever medication¹
- ↑Higher dose of medication required to reach asthma control²
- ↑Resistance to steroid treatment²
- ↑Exercise-related asthma¹
- ↓Impaired lung function (FVC, FEV₁)¹
- ↓Worse asthma control³



1. Ali, 2013. *Respiratory Medicine* 107, 1287-1300

2. Forgie, 2011. *J Allergy Clin Immunol*. Mar;127(3):741-8. doi: 10.1016/j.jaci.2010.12.010

3. Quinto, 2011. *J Allergy Clin Immunol*. Nov;128(5):964-8. doi: 10.1016/j.jaci.2011.06.031

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Conclusion & Future Implications

High BMI in asthmatic children was associated with increased lung volume (FVC) and airflow (FEV₁) along with reduced FeNO in overweight/obese.

This is significant because pulmonary function tests may have reduced sensitivity in asthmatic children with increased BMI.

Future research is needed to establish whether disproportionate lung growth and non-eosinophilic bronchial inflammation might be the underlying mechanisms for this paradox.



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Oral Presentations (presented in Greek)

9. 14th PanHellenic Nutrition & Dietetics (HDA) Conference, Nov 2017

14ο Πανελλήνιο Συνέδριο Διατροφής Διαταραχές
24-26 Νοεμβρίου, 2017 Αθήνα

Η επίδραση της Μεσογειακής Διατροφής εμπλουτισμένη με λιπαρό ψάρι στη διαχείριση του παιδικού άσθματος: Τυχαιοποιημένη κλινική μελέτη

M.M. Παπαμιχαήλ¹ PhD; X. Κατσαρός² MD PhD; Δ. Τσουκαλάς³ MD; B. Erbas² PhD; K. Ισιγόπουλος⁴ PhD

¹La Trobe University, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia.
²La Trobe University, Department of Public Health, Melbourne, Australia.
³National & Kapodistrian University of Athens, Athens, Greece
⁴European Institute of Nutritional Medicine, Rome, Italy

Παιδικό άσθμα

- Το άσθμα είναι η πιο συχνή ασθένεια των παιδιών, **αυξάνεται** συνεχώς **παγκόσμιως**.⁽¹⁾
- Είναι ένας από τους συνηθέστερους λόγους για:
- Επίσκεψη σε ιδιωτικό ιατρείο, νοσοκομείο ή στα επείγοντα⁽¹⁾
- Απουσία των παιδιών από το σχολείο και των γονέων τους από την εργασία τους⁽¹⁾
- Περιορισμός τις καθημερινές δραστηριότητες του παιδιού
- Μείωση της ποιότητας ζωής (QOL) για όλη την οικογένεια
- Υψηλό οικονομικό κόστος για το Σύστημα Υγείας και για τον ασθενή λόγω της ιατρικής περίθαλψης και της φαρμακευτικής αγωγής
- Έχει υπολογιστεί ότι στα παιδιά (5-17 ετών), περίπου **10,000 ημέρες** ανα έτος χάνονται από το σχολείο λόγω του άσθματος⁽²⁾.

1. WHO, 2017. Asthma <http://www.who.int/mediacentre/factsheets/fs207/en/>
2. Global Initiative for Asthma (GINA), 2017. <http://ginasthma.org/2017-pocket-guide-for-asthma-management-and-prevention/>

Ελλάδα **Παιδικό άσθμα**

- Στην Ελλάδα, 11% των παιδιών πάσχουν από άσθμα (≈ 1 στα 10)⁽¹⁾
- 50-80% των ασθματικών παιδιών εκδηλώνουν συμπτώματα άσθματος πριν την ηλικία των 5 ετών⁽²⁾
- Υπερτερεί στα αγόρια σε μικρές ηλικίες και στα κορίτσια μετά τη εφηβεία
- Σε 50% των παιδιών το άσθμα παραμένει στην ενήλικη ζωή⁽³⁾

1. <http://www.ginasthma.gr>
2. WHO, 2017. Retrieved from <http://www.who.int/mediacentre/factsheets/fs207/en/>
3. Global Initiative for Asthma (GINA), 2017. <http://ginasthma.org/2017-pocket-guide-for-asthma-management-and-prevention/>

Παθοφυσιολογία

➤ Το άσθμα είναι πολυπαράγοντική ασθένεια που προκαλείται από γενετικούς και περιβαλλοντικούς παράγοντες⁽¹⁾

Κληρονομικότητα + Περιβαλλοντικοί παράγοντες

Θεραπεία

- Συμπτώματα ελέγχονται μόνο με τη φαρμακευτική αγωγή⁽²⁾

1. GINA, 2017. <http://ginasthma.org/2017-pocket-guide-for-asthma-management-and-prevention/>

Μεθοδολογία Αντιμετώπισης
Συστηματική Ανασκόπηση

Public Health Nutrition paper 1 of 13 doi:10.1017/S136900017001823

Review Article

Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? A systematic review of observational studies

Maria M Papamichael¹*, Catherine Isiopoulos¹, Nugroho H Susanto² and Biran Erbas²
¹School of Allied Health, Department of Rehabilitation, Nutrition and Sport, La Trobe University, Melbourne, VIC 3086, Australia; ²School of Psychology and Public Health, Department of Public Health, La Trobe University, Melbourne, Australia

Scheduled 23 January 2017. Final version received 26 May 2017. Accepted 19 June 2017

Συμπεράσματα

Συνολικά, η συστηματική ανασκόπηση αποκάλυψε ότι **υψηλή προσκόλληση στη Μεσογειακή διατροφή μείωσε τα συμπτώματα άσθματος (1) στα παιδιά** δηλαδή δρα προστατευτικά.

Papamichael MM, Isiopoulos C, Erbas B, Susanto NH. 2017. Public Health Nutr. Aug 14:1-13. doi: 10.1017/S136900017001823.

Μεθοδολογία Αντιμετώπισης
Η κατανάλωση ψαριού;

- Επιδημιολογικές μελέτες αναφέρουν ότι η κατανάλωση ψαριού (τουλάχιστον 1/εβδομάδα) μείωσε κατά 27-80% τον κίνδυνο του άσθματος στα παιδιά⁽¹⁾.
- Οι Hodge et al, βρήκαν ότι η κατανάλωση λιπαρών ψαριών σε σχέση με οποιοδήποτε ψάρι είχε μεγαλύτερη μείωση του κινδύνου του άσθματος κατά 74% έναντι 48%⁽²⁾.

1. Koppelman LS, et al. 2011. Clin Rev Allergy Immunol. 41(2):34-66.
2. Hodge L, et al. 1996. Aust J Med. 1996 Feb 5; 164(3):137-46.

Μεθοδολογία Αντιμετώπισης

Η επίδραση της Μεσογειακής διατροφής εμπλουτισμένη με λιπαρό ψάρι στη διαχείριση του παιδικού άσθματος: Τυχαιοποιημένη Κλινική Μελέτη

Protocol No: ACTRN12616000492459p

Σκοπός

Να ερευνηθεί αν η κατανάλωση λιπαρών ψαριών στο πλαίσιο της Μεσογειακής Διατροφής μειώνει τα συμπτώματα στα παιδιά που πάσχουν από άσθμα στην Ελλάδα.

Μεθοδολογία Αντιμετώπισης
Ταυτότητα της έρευνας

- **Είδος Μελέτης:** Τυχαιοποιημένη κλινική μελέτη (Parallel Randomized Controlled Trial)
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- **Τόπος:** Ιδιωτική παιδιατρική κλινική άσθματος, Αθήνα
- **Διάρκεια:** 6 μήνες
- **Sample size calculation⁽¹⁾:**
Μέγεθος του δείγματος (n) = **64** (90% power, E.F = 0.4, α = 5%, drop out = 20%)
32 συμμετέχοντες ανά ομάδα.
- **Ομάδες:** 1. **Παρέμβαση:** 2 φ/εβδομάδα (τουλάχιστον 150 γρ. μαγειρεμένο λιπαρό ψάρι/γεύμα) στο πλαίσιο της Μεσογειακής διατροφής⁽²⁾
2. **Ελέγχου:** η κατανάλωση της συνήθους διατροφής τους

1. Lee et al. 2013. BMC. 110:145-155
2. PROLEPSIS 2017. <http://www.diastrophiloiologi.gr/?page=diastrophiloi-oligi-paidia-systasis>

10.12th PanHellenic College of Pediatrics Conference, Feb 2-4 2018, Athens, Greece

12th ΠΑΝΕΛΛΗΝΙΟ ΣΥΝΕΔΡΙΟ ΕΛΛΗΝΙΚΟΥ ΚΟΛΛΕΓΙΟΥ ΠΑΙΔΙΑΤΡΩΝ
3rd ΠΑΝΕΛΛΗΝΙΟ ΣΥΝΕΔΡΙΟ ΕΛΛΗΝΙΚΗΣ ΕΤΑΙΡΕΙΑΣ ΠΑΙΔΙΚΗΣ & ΕΦΗΒΙΚΗΣ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑΣ
2 - 4 ΦΕΒΡΟΥΑΡΙΟΥ 2018, ΑΘΗΝΑ

Η επίδραση της Μεσογειακής Διατροφής εμπλουτισμένη με λιπαρό ψάρι στο παιδικό άσθμα. Μια τυχαιοποιημένη κλινική μελέτη (RCT)

M.M. Παπαμιχαήλ¹, Ph.D; Δ. Τσουκαλάς², M.D; B.Erbas², Ph.D; K. Ιτατόπουλος², Ph.D; M. Κουτσούμης¹, Ph.D; X. Κατσαράκης², MD Ph.D

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1. J Pharmacy & Pharmacology Feb 2018. In Press

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1. Lee et al. 2015. BMJ 350:f45-155
2. PREDICTED, 2017. <http://www.predicted.gr/?page=diastrophis-edra-pedio-astmatas>

20. 15th PanHellenic Nutrition & Dietetics Conference Dec 13-15, 2019 Athens, Greece

ΜΠΟΡΕΙ ΜΙΑ ΜΕΣΟΓΕΙΑΚΗ ΔΙΑΤΡΟΦΗ ΕΜΠΛΟΥΤΙΣΜΕΝΗ ΜΕ ΛΙΠΑΡΟ ΨΑΡΙ ΝΑ ΕΠΗΡΕΑΣΕΙ ΤΗΝ ΠΝΕΥΜΟΝΙΚΗ ΛΕΙΤΟΥΡΓΙΑ ΣΤΑ ΑΣΘΜΑΤΙΚΑ ΠΑΙΔΙΑ;

Μαρία Μισέλ Παπαμιχαήλ¹, Χάρης Κατσαρδής³, Katrina Lambert², Δημήτρης Τσουκαλάς⁴, Bircan Erbas², Κατερίνα Ισιόπουλος^{1,5}

¹ Πανεπιστήμιο La Trobe, Σχολή Επιστημών Υγείας, Μελβούρνη, Αυστραλία.
² Πανεπιστήμιο La Trobe, Σχολή Δημόσιας Υγείας, Μελβούρνη, Αυστραλία.
³ Εθνικό & Καποδιστριακό Πανεπιστήμιο Αθηνών, Ελλάδα
⁴ Ευρωπαϊκού Ινστιτούτου Διατροφικής Ιατρικής, Ρώμη, Ιταλία
⁵ Πανεπιστήμιο Murdoch, Σχολή Επιστημών Υγείας, Μηχανικών και Εκπαίδευσης, Πέρθ, Αυστραλία



Εισαγωγή

- Το άσθμα έχει αυξηθεί δραματικά παγκοσμίως στα παιδιά τα τελευταία 30 χρόνια^{1,2}
- Στην Ελλάδα ~ 1 στα 10 παιδιά πάσχει από άσθμα³
- Συνδέεται με αυξημένη νοσηρότητα, μερικές φορές θνησιμότητα και κακή ποιότητα ζωής^{1,2}
- Είναι ένας από τους συνηθέστερους λόγους για τη νοσηλεία των παιδιών, τις επισκέψεις στο παιδίατρο, την απουσία από το σχολείο και την εργασία των γονέων^{1,2}
- Εμφανίζεται νωρίς στη ζωή (~ 6 ετών), συχνότερα στα αγόρια και επικρατεί στα κορίτσια κατά τη εφηβεία¹
- Το 50% των παιδιών το άσθμα παραμένει στην ενήλικη ζωή¹
- Συμφώνη με ΠΟΥ, ~ 10 εκατομμύρια σχολικές ημέρες/χρόνο χάνονται από το σχολείο λόγω άσθματος¹
- Επίσης προκαλεί οικονομική επιβάρυνση για τον ασθενή και την κοινωνία λόγω νοσηλείας και κόστους φαρμακευτικής αγωγής¹

1. WHO, 2017. Asthma Fact sheet. Available from <http://www.who.int/mediacentre/factsheets/fs307/en/>
 2. Nunes et al. Asthma Research and Practice (2017) 3:2
 3. <http://www.mysasthma.gr>

Η Κλινική Δοκιμή- RCT

Σκοπός

- Διενεργήσαμε εξάμηνη κλινική δοκιμή για να διερευνήσουμε την αποτελεσματικότητα της Μεσογειακής Διατροφής στη βρογχική φλεγμονή στα ασθματικά παιδιά^{1,2}.

Protocol No: ACTRN12616000492459p//www.anzctr.org.au

1. Papamichael et al, 2019. J Hum Nutr & Dietetics (32): 185-197. <http://doi.org/10.1111/jhn.12609>
 2. Papamichael et al, 2018. J Pharmacy & Pharmacology Feb, 6 (2018) 225-239. doi: 10.17265/2328-2150/2018.03.004

Μεθοδολογία/Υλικά

- Τυχαιοποιημένη ελεγχόμενη κλινική δοκιμή (RCT)
- 72 παιδιά (5-12 ετών) με ιατρό-διαγνωσμένο 'ήπιο' άσθμα επιλέχθηκαν από ένα παιδιατρικό ιατρείο άσθματος στην Αθήνα από Νοέμ-Δεκ 31, 2016
- Τυχαιοποιήθηκαν σε 2 ομάδες:

1. Ομάδα παρέμβασης (n=36):
 Κατανάλωνε μια Μεσογειακή διατροφή εμπλουτισμένη με 2 γεύματα λιπαρού ψαριού*/εβδομάδα (≥150gr μαγειρεμένο φιλέτο/γεύμα) για έξι μήνες^{1,2}
 *λιπαρά ψαριά- Σολωμός, πέστροφα, σαρδέλα, γαύρο, κολιός, σκουμπρί, τσιπούρα

2. Ομάδα ελέγχου (n=36): τη συνήθη διατροφή της

Ref: 1. Hellenic Ministry of Health & Welfare, 1999. Archives of Hellenic Medicine, 1999; 16(3):316-324
 2. PROLEPSIS, 2016. <http://www.diattrofikioidioidi.gr/?page=diattrofikioidioidi>

Εισαγωγή

- Το άσθμα ορίζεται ως μια χρόνια **φλεγμονώδης διαταραχή των αεραγωγών** που χαρακτηρίζεται από επεισόδια συριγμού, δύσπνοια, βήχας και σφίξιμο στο θώρακα ειδικά τη νύχτα και νωρίς το πρωί^{1,2}.
- Η Παραδοσιακή Μεσογειακή Διατροφή είναι μια **αντιφλεγμονώδης και αντιοξειδωτική διατροφή** λόγω της υψηλής περιεκτικότητας σε λαχανικά, φρούτα, ελαιόλαδο και ψάρι που είναι πλούσια σε αντιοξειδωτικά και οмега-3 λιπαρά οξέα^{3,4}.

Ref: 1. Papadopoulos et al, 2012. Allergy 67 (2012) 976-997
 2. Doering, 2013. J Acad Physiol (1985) 2013 Apr 1; 114(7): 834-843
 3. Calder, 2013. Biochim Biophys Acta. Apr;1831(4):469-84.
 4. Simopoulos, 2002. Nutr. 131: 3065S-3073S, 2001.

BDA The Association of UK Dietitians
Journal of Human Nutrition and Dietetics
 THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION
 Journal of Human Nutrition and Dietetics

ALLERGY AND ATOPY
Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial
 M. M. Papamichael,¹ Ch. Katsaridis,² K. Lambert,³ D. Tsoukalas,⁴ M. Koutisieris,² B. Erbas³ & C. Isiopoulos¹

Journal of Pharmacy and Pharmacology 6 (2019) 225-239
 doi: 10.17265/2328-2150/2018.03.004

A Clinical Trial of Mediterranean Diet Enriched with Fatty Fish in Pediatric Asthma: Study Protocol

Maria Michela Papamichael¹, Charis Katsaridis², Desmon Tsoukalas³, Bircan Erbas³ and Catherine Isiopoulos¹
¹ La Trobe University, School of Clinical Health, Department of Rehabilitation, Nutrition & Sport, Melbourne 3086, Australia
² La Trobe University, School of Psychology & Public Health, Department of Public Health, Melbourne 3086, Australia
³ National & Kapodistrian University of Athens, Athens 11527, Greece
⁴ European Institute of Nutritional Medicine, Rome 00198, Italy

Αποτελέσματα

Πίνακας 3. Μεταβολή στη μέση τιμή του eNO

Mean score change FeNO(ppb)	Variable	Difference		p*
		β	95% CI	
	Group	-14.15	-27.39, -0.91	0.037
	Age	-1.69	-4.97, 1.59	0.31
	Sex	6.89	-6.23, 20.10	0.30
	Regular physical activity	1.69	-11.51, 14.89	0.80
	BMI	0.22	-1.64, 2.09	0.81

β- unstandardized beta
 P* Multiple regression adjusting for confounders (age, sex, BMI, regular physical activity)

Το μοντέλο γραμμικής παλινδρόμησης προσαρμοσμένο στην ηλικία, το φύλο, τη φυσική δραστηριότητα και το BMI έδειξε ότι το FeNO μειώθηκε κατά 14 ppb από την αρχική τιμή στην ομάδα παρέμβασης σε σύγκριση με την ομάδα ελέγχου, (p= 0.04, β= - 14.15 ppb, 95%CI: - 27.39, -0.91).

Δεν βρέθηκε στατιστική σημαντική μεταβολή στη μέση τιμή των παραμέτρων της σπυρομέτρησης (p>0.05)

21. Annual Conference of the College of Pediatrics Feb 14-15th, 2020, Athens, Greece

Ετήσιο Συνέδριο Επιστημονική Κοινότητα Παιδιάτρων
14-15 Φεβρουαρίου 2020
Grand Ballroom Park Hotel

Η ΕΠΙΔΡΑΣΗ ΤΟΥ ΣΩΜΑΤΙΚΟΥ ΒΑΡΟΥΣ ΣΤΗΝ ΠΝΕΥΜΟΝΙΚΗ ΛΕΙΤΟΥΡΓΙΑ ΤΩΝ ΑΣΘΜΑΤΙΚΩΝ ΠΑΙΔΙΩΝ

Παπαμιχαήλ Μαρία Μισέλ¹; Τσουκαλάς Δημήτρης⁴; Erbas Bircan²; Ισιόπουλος Κατερίνα^{1,5}; Κατσαρδής Χαράλαμπος³

¹ Πανεπιστήμιο La Trobe, Σχολή Επιστημών Υγείας, Μελβούρνη, Αυστραλία.
² Πανεπιστήμιο La Trobe, Σχολή Δημόσιας Υγείας, Μελβούρνη, Αυστραλία.
³ Εθνικό & Καποδιστριακό Πανεπιστήμιο Αθηνών, Ελλάδα
⁴ Ευρωπαϊκό Ινστιτούτο Διατροφικής Ιατρικής, Ρώμη, Ιταλία
⁵ Πανεπιστήμιο Murdoch, Σχολή Επιστημών Υγείας, Μηχανικών και Εκπαίδευσης, Πέρθ, Αυστραλία

Εισαγωγή

- Το άσθμα έχει αυξηθεί δραματικά παγκοσμίως στα παιδιά τα τελευταία 30 χρόνια ^{1,2}
- Στην Ελλάδα, τουλάχιστον 1 στα 10 παιδιά πάσχει από άσθμα ³
- Συνδέεται με αυξημένη νοσηρότητα, μερικές φορές θνησιμότητα και κακή ποιότητα ζωής ^{1,2}
- Είναι ένας από τους συνηθέστερους λόγους για τη νοσηλεία των παιδιών, τις επισκέψεις στο παιδίατρο, την απουσία από το σχολείο και την εργασία των γονέων ^{1,2}

1. WHO, 2017. Asthma Fact sheet. Available from <http://www.who.int/mediacentre/factsheets/fs307/en/>
2. Nunes et al. Asthma Research and Practice (2017) 3:1
3. <http://www.mayoclinic.org>

Γονίδια και Περιβαλλοντικοί Παράγοντες^{1,2}

1. GINA, 2016. <http://www.ginasthma.com>
2. Ober, 2011. [immunol.org](http://www.immunol.org) 2011 Jul; 242(1): 10-30.

Η επίδραση του υπερβολικού βάρους vs φυσιολογικού στα ασθματικά παιδιά

- ↑ Καθημερινά συμπτώματα άσθματος¹
- ↑ Νοσηλεία¹
- ↑ Απουσίες από το σχολείο¹
- ↑ Λήψη φαρμάκων για το άσθμα^{1,2}
- ↑ Αντίσταση στα στεροειδή²
- ↑ Άσθμα που σχετίζεται με την άσκηση¹
- ↓ Λειτουργία των πνευμόνων (FVC, FEV₁)¹
- ↓ Έλεγχος του άσθματος³

1. Ali, 2013. Respiratory Medicine 107, 1287-1300
2. Forno, 2011. J Allergy Clin Immunol. Mar; 127(3):741-9. doi: 10.1016/j.jaci.2010.12.010
3. Quinto, 2011. J Allergy Clin Immunol. Nov; 128(5):964-9. doi: 10.1016/j.jaci.2011.06.031.

FeNO χαμηλότερο στους υπέρβαρους/παχύσαρκους σε σύγκριση με το φυσιολογικό βάρος!

*p=0.03
*p-value Mann-Whitney test

Σχήμα 4. Διαφορά στο διάμεσο FeNO ανά κατηγορία του ΔΜΣ

Πίνακας 2. Μοντέλο γραμμικής παλινδρόμησης

Παράμετρος Σπυρομέτρησης	Συν-μεταβλητές	β	95%CI	P-value ^a
FEF _{25-75%} (% predicted)	Ομάδα ΔΜΣ (υπέρβαρο/παχύσαρκο vs φυσιολογικό βάρος)	11.65	0.36, 22.94	0.043
	Ηλικία	3.59	-2.10, 9.27	0.21
	Υψος	-0.76	-1.76, 0.23	0.13
	Φύλο	-4.42	-14.65, 5.79	0.39
	Φυσική δραστηριότητα ($\geq 3X/\epsilon 85$)	-3.67	-14.21, 6.87	0.49
	Φαρμακευτική Αγωγή	3.74	-10.52, 18.00	0.60
	Συμμόρφωση στη Μεσογειακή Διατροφή (KIDMED σκορ)	-1.64	-4.19, 0.90	0.20

Κλειδί: FEF_{25-75%}=Mid Expiratory Flow 25-75% vital capacity
*Σπυρομέτρηση (% predicted) πριν βρογχοδιασταλτικό.
 β -Unstandardized coefficient; 95%CI=95% Confidence Interval
^aP-Μοντέλο γραμμικής παλινδρόμησης προσαρμοσμένο σε ηλικία, ύψος, φύλο, φυσική δραστηριότητα, φάρμακα και προσκόλληση στη Μεσογειακή Διατροφή (μέσω KIDMED σκορ).

Σύνοψη

Στο παιδικό άσθμα ο υψηλός ΔΜΣ συσχετίστηκε με αυξημένη ροή αέρα στους κεντρικούς και περιφερειακούς αεραγωγούς, όπως αποδεικνύεται από τις παραμέτρους σπυρομέτρησης FEV₁, FVC και FEF_{25-75%} και το μειωμένο FeNO σε υπέρβαρο/παχύσαρκα παιδιά. Αυτό είναι σημαντικό, επειδή η σπυρομέτρηση και το FeNO μπορεί να έχουν μειωμένη ευαισθησία σε ασθματικά παιδιά με αυξημένο ΔΜΣ. Απαιτούνται μελλοντικές μελέτες για να διαπιστώσουν αν οφείλεται σε δυσανάλογη ανάπτυξη πνευμόνων και διαμέτρου των αεραγωγών (δυσανάπτυξη) και στη μη ηωσινοφιλική φλεγμονή των αεραγωγών.

Συμπέρασμα

Η παιδική παχυσαρκία συνδέεται με σοβαρές συνέπειες για την υγεία, συμπεριλαμβανομένου του άσθματος. Οι επαγγελματίες υγείας πρέπει να συστήνουν μια υγιεινή ισορροπημένη διατροφή (όπως η Μεσογειακή Διατροφή) και καθημερινή σωματική δραστηριότητα για τη διαχείριση του υπέρβαρου/παχύσαρκας σε παιδιατρικούς ασθενείς με άσθμα.

Awards

9.14th Pan Hellenic Nutrition & Dieticians Conference (HDA), November 24-27 2017, Athens Greece (English translation)

From Evi Mitrogianni emitrogianni@goldair.gr

RE: 14th PanHellenic Nutrition & Dietetic Conference (HDA AWARD) 2017

Date: 29.11.17

Dear Mrs. Papamichael,

We would like to inform you that the study titled “A clinical trial on the efficacy of a Mediterranean diet enriched with fatty fish in the management of paediatric asthma: Preliminary results.” has received an award at the 14th Hellenic Nutrition & Dietetic Conference/ 3rd Hellenic Clinical Nutrition and Metabolism Conference.

Please send us your postal address so that we may send you the certificate.

Yours Sincerely

Evi Mitrogianni|Congress Coordinator

1st Km, Peanias Markopoulou Ave | 19002 Peania, Greece ([map](#))

T : +30 210 3274694 | emitrogianni@goldair.gr

F : +30 210 3311021 | www.goldaircongress.gr

Iata Member. Numeric code : 27 – 2 1336 2

Goldair is an ISO 9001:2008 and IQnet certified company

ICCA, HAPCO & ACVB Member

9.14th Pan Hellenic Nutrition & Dieticians Conference (HDA), November 24-27 2017, Athens Greece (Greek)

From: Evi Mitrogianni emitrogianni@goldair.gr

Date 29.11.2017

Αγαπητή Κ.Παπαμιχαήλ,

Θα θέλαμε να σας ενημερώσουμε ότι η εργασία σας με τίτλο “ ΚΛΙΝΙΚΗ ΔΟΚΙΜΗ ΣΧΕΤΙΚΑ ΜΕ ΤΗΝ ΕΠΙΔΡΑΣΗ ΤΗΣ ΜΕΣΟΓΕΙΑΚΗΣ ΔΙΑΤΡΟΦΗΣ ΕΜΠΛΟΥΤΙΣΜΕΝΗ ΜΕ ΛΙΠΑΡΟ ΨΑΡΙ ΣΤΗ ΔΙΑΧΕΙΡΙΣΗ ΤΟΥ ΠΑΙΔΙΚΟΥ ΑΣΘΜΑΤΟΣ: ΠΡΟΚΑΤΑΡΚΤΙΚΑ ΑΠΟΤΕΛΕΣΜΑΤΑ ”, έλαβε έπαινο στο *14^ο Πανελλήνιο Συνέδριο διατροφής και διαιτολογίας και 3^ο Πανελλήνιο Συνέδριο κλινικής διατροφής και μεταβολισμού.*

Παρακαλώ ενημερώστε μας για τα στοιχεία της ταχυδρομικής διεύθυνσης που θα θέλατε να σας σταλεί ο έπαινος.

Με εκτίμηση

Evi Mitrogianni|Congress Coordinator

1st Km, Peanias Markopoulou Ave | 19002 Peania, Greece ([map](#))

T : +30 210 3274694 | emitrogianni@goldair.gr

F : +30 210 3311021 | www.goldaircongress.gr

Iata Member. Numeric code : 27 – 2 1336 2

Goldair is an ISO 9001:2008 and IQnet certified company

ICCA, HAPCO & ACVB Member

9. 14th Pan Hellenic Nutrition & Dieticians Conference (HDA), AWARD



14th Hellenic Nutrition & Dietetics Conference/3rd Hellenic Clinical Nutrition & Metabolism Conference 24-26 November 2017.

AWARD

We would like to inform that the Oral presentation titled:

**A clinical trial on the efficacy of a Mediterranean diet enriched with fatty fish in
the management of paediatric asthma: Preliminary results.**

Maria Papamichael, Charis Katsardis, Dimitrios Tsoukalas, Bircan Erbas, Catherine Itsiopoulos

has been awarded with ‘Distinction’ at the **14th Hellenic Nutrition & Dietetics Conference/3rd Hellenic Clinical Nutrition & Metabolism Conference** which was held on 24-26 November 2017 at “Theatro” Cultural Centre and “Hellenic World”, Athens Greece.

Signed: Organizing/Scientific Committee/Hellenic Clinical Nutrition & Metabolism Association/Hellenic Nutrition and Dietetics Association. www.hdacongress.gr

APPENDIX 5 MEDIA RELEASE 2018 AUSTRALIA

Diet rich in fish helps fight asthma

4 November 2018



A clinical trial led by La Trobe University has shown eating fish such as salmon, trout and sardines as part of a healthy diet can reduce asthma symptoms in children.

The international study found children with asthma who followed a healthy Mediterranean diet enriched with **fatty fish** had improved lung function after six months.

Lead researcher Maria Papamichael from La Trobe said the findings added to a growing body of evidence that a healthy diet could be a potential therapy for **childhood asthma**.

"We already know that a diet high in fat, sugar and salt can influence the development and progression of asthma in children and now we have evidence that it's also possible to manage asthma symptoms through healthy eating," Ms Papamichael said.

"Fatty fish is high in **omega-3 fatty acids** which have anti-inflammatory properties. Our study shows eating fish just twice a week can significantly decrease lung inflammation in children with asthma."

Co-researcher and Head of La Trobe's School of Allied Health, Professor Catherine Itsiopoulos, said the results were promising.

"Following a traditional Mediterranean diet that is high in plant-based foods and oily fish could be an easy, safe and effective way to reduce **asthma symptoms** in children," Professor Itsiopoulos said.

Associate Professor Bircan Erbas, from La Trobe's School of Psychology and Public Health, is an expert in asthma and allergies, who co-supervised the trial.

"Asthma is the most common respiratory disease in young people and one of the leading reasons for hospitalisations and trips to emergency for children," Associate Professor Erbas said.

"Unfortunately, the rate of asthma worldwide remains high. It is imperative that we identify new therapies that we can use alongside conventional asthma medications."

The clinical trial involved 64 children from Athens in Greece, aged 5 to 12 who had mild **asthma**. Researchers from Australia and Greece divided the children into two groups and instructed around half to eat two meals of cooked fatty fish (of at least 150 grams) as part of the Greek Mediterranean diet every week for six months. The remaining children followed their normal diet.

At the end of the trial, they found the group who ate fish had reduced their bronchial inflammation by 14 units. Above 10 units is significant under international guidelines.

Read the study's findings in the *Journal of Human Nutrition and Dietetics*.

More information: M. M. Papamichael et al. Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial, *Journal Human Nutrition & Dietetics* (2018).DOI: 10.1111/jhn.1260

Provided by La Trobe University

APA citation: Diet rich in fish helps fight asthma (2018, November 4) retrieved 5 November 2018 from <https://medicalxpress.com/news/2018-11-diet-rich-fish-asthma.html>.
Published in Herald-Sun November 5th 2018

Links:

1. **La Trobe University:** <https://www.latrobe.edu.au/news/articles/2018/release/diet-rich-in-fish-helps-fight-asthma>
2. **10 News Melbourne:** <https://tenplay.com.au/news/melbourne/2018/11/5/asthma-sufferers-may-be-able-to-control-condition-through-eating-more-fish>
3. **9 News Melbourne:** <https://www.9news.com.au/videos/national/fish-could-reduce-asthma-symptoms/cjo3u5q7x000u0qmf0t3st7gi>
4. **Huffington Post UK:** https://www.huffingtonpost.co.uk/entry/diet-rich-in-fatty-fish-can-reduce-asthma-symptoms-in-children-study-suggests_uk_5be16318e4b04367a88029ac?utm_hp_ref=uk-parents&guccounter=1&guce_referrer_us=aHR0cHM6Ly93d3cuZ29vZ2xllmdyLw&guce_referrer_cs=m8lpbEMeR1I46h-il9PA5w
5. **Eurek Alert, American Association for the Advancement of Science:**
https://www.eurekalert.org/pub_releases/2018-11/ltu-tfd110218.php
6. **Medical Express:** <https://medicalxpress.com/news/2018-11-diet-rich-fish-asthma.html>
7. **Medi Bulletin:** <https://medibulletin.com/fatty-fishes-like-salmon-trout-sardines-help-kids-fight-asthma/>
8. **Economic Times, Health:**
<https://health.economictimes.indiatimes.com/news/diagnostics/diet-rich-in-fish-helps-fight-asthma-study/66510832>
9. **Reddit Science:**
https://www.reddit.com/r/science/comments/9ubt7h/diet_rich_in_fish_helps_fight_asthma_children/
10. **AJC:**
<https://www.ajc.com/news/health-med-fit-science/can-diet-rich-fish-help-fight-childhood-asthma/R0CV9MfN6OOcLCIsXsdWYO/>
11. **Sydney Strait Times:** <https://www.nst.com.my/lifestyle/heal/2018/11/428527/fish-consumption-reduces-symptoms-childhood-asthma-study>
12. **Genetic Engineering and Biotechnology News:**
<https://www.genengnews.com/news/oily-fish-reduces-airway-inflammation-in-asthmatic-children/>
13. **The Cordova Times Alaska:**
<https://www.thecordovaitimes.com/2018/11/06/study-fish-rich-diet-helps-fight-asthma/>

14. Pharmacy News:

<https://www.pharmacynews.com.au/news/why-eating-more-fish-can-help-children-asthma-0>

15. BHG News:

<https://www.bhg.com.au/diet-rich-in-fatty-fish-could-reduce-asthma-symptoms-in-children>

16. Pulmonology Advisor:

<https://www.pulmonologyadvisor.com/home/topics/asthma/fatty-fish-and-mediterranean-diet-effective-as-asthma-treatment-in-children/>

17. Science Daily: <https://www.sciencedaily.com/releases/2018/11/181104085114.htm>**18. Cordova Times:** <https://www.thecordovatimes.com/2018/11/06/study-fish-rich-diet-helps-fight-asthma/>**19. Pittwater News:** <http://www.pittwateronlinenews.com/Inbox-and-Environment-News-Issue-383.php>**20. Medical Net News:** <https://www.news-medical.net/news/20181105/Trial-shows-fish-enriched-diet-may-reduce-childhood-asthma-symptoms.aspx>**21. Metabolomics Medicine Greece:**

https://www.metabolomicmedicine.com/diethnis_dimosiotita_sti_meleti_mas_gia_to_asthma_kai_ti_diatrofi-na-212.html

22. Metabolomics Medicine Greece:

https://www.drtsoukalas.com/paidiko_asthma_kai_mesogeiki_diatrofi-su-218.html

23. Insurance world Greece: <https://insuranceworld.gr/54874/eidiseis/diethnis-dimosiotita-sti-meleti-mas-gia-to-asthma-kai-ti-diatrofi/>**24. BHMA Newspaper Greece** https://www.tovima.gr/printed_post/sardela-kai-gayros-lfenantion-asthmatos/

APPENDIX 5

MEDIA PRESS RELEASE NOVEMBER 2018: GREECE

Newspaper BHMA November 25th 2018 (printed and online)



Παιδιατρική

Σαρδέλα και γαύρος εναντίον άσθματος

Μείωση της λήψης εισπνεόμενων παρατηρήθηκε σε παιδιά που κατανάλωναν δύο ψαρογεύματα την εβδομάδα στο πλαίσιο της μεσογειακής διατροφής

Τσώλη Θεοδώρα

| 25.11.2018 - 08:00

Είναι τα παρεξηγημένα, τα θεωρούμενα ως δεύτερης διαλογής στο ψαράδικο: είναι η σαρδέλα, ο γαύρος, το σκουμπρί, ο κολιός. Τα «ταπεινά» όμως αυτά λιπαρά ψάρια αποτελούν «βόμβες» θρεπτικών συστατικών και κυρίως ω-3 λιπαρών οξέων τα οποία έχουν πολλαπλά οφέλη για τον οργανισμό. Όπως δείχνει μάλιστα νέα μελέτη που δημοσιεύθηκε πρόσφατα (συγκεκριμένα στις 30 Οκτωβρίου) στην επιθεώρηση «Journal of Human Nutrition and Dietetics», τα λιπαρά ψάρια σαν και αυτά που σας αναφέραμε δεν είναι μόνο «βάλσαμο» για την τσέπη μας (ειδικά την περίοδο της οικονομικής κρίσης) αλλά μπορεί να αποδειχθούν και «φάρμακο» ενάντια σε μία από τις πιο συχνές χρόνιες νόσους, και δη των μικρών παιδιών, το άσθμα.

Ψάρια αντί φαρμάκων;

Το άσθμα που προκαλεί φλεγμονή των βρόγχων αποτελεί σημαντική αιτία καθημερινής νοσηρότητας των παιδιών, εισαγωγών τους στα νοσοκομεία, επισκέψεων σε παιδιάτρους ενώ

αποτελεί και τον υπ' αριθμόν 1 ένοχο για την απουσία των μαθητών από το σχολείο. Ένα στα 10 παιδιά στην Ελλάδα πάσχουν από σοβαρό άσθμα με αποτέλεσμα να ζουν με μόνιμο «σύντροφο» τα εισπνεόμενα, κυρίως κορτικοστεροειδή, φάρμακα. Φανταστείτε λοιπόν πόσο σημαντικό θα ήταν ένα φθηνό ψαράκι σερβιρισμένο στο μεσημεριανό τραπέζι να προσφέρει μείωση της βρογχικής φλεγμονής αλλά και μείωση της λήψης φαρμάκων από ασθματικά παιδιά!

Αυτό λίγο ως πολύ έδειξε η νέα μελέτη, πρώτη συγγραφέας της οποίας ήταν η κλινική διαιτολόγος, ερευνήτρια και υποψήφια διδάκτωρ στο Πανεπιστήμιο La Trobe στη Μελβούρνη της Αυστραλίας κυρία **Μαρία Παπαμιχαήλ**. Η μελέτη αποτελεί μέρος της διδακτορικής διατριβής που εκπονεί η κυρία Παπαμιχαήλ με επιβλέπουσα την καθηγήτρια του La Trobe κυρία **Κατερίνα Ιτσιόπουλος**, η οποία ασχολείται ερευνητικά με τα οφέλη της μεσογειακής διατροφής και της επεκτασιμότητάς της σε πληθυσμούς εκτός Μεσογείου για περισσότερο από 20 χρόνια, ενώ στη διεξαγωγή της συνεργάστηκαν επίσης ο επίκουρος καθηγητής Παιδοπνευμονολογίας του Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών κ. **Χαράλαμπος Κατσαρδής** και ο δρ **Δημήτρης Τσουκαλάς** του Ευρωπαϊκού Ινστιτούτου Διατροφικής Ιατρικής στη Ρώμη.

Μείωση φλεγμονής

‘Όπως ανέφερε η κυρία Παπαμιχαήλ στο «Βήμα», «μελέτες που έχουν διεξαχθεί ως σήμερα σε μοριακό επίπεδο αλλά και σε ζωικά μοντέλα έχουν δείξει ότι τα ω-3 λιπαρά οξέα που περιέχονται στα λιπαρά ψάρια μειώνουν τη βρογχική φλεγμονή η οποία αποτελεί το κύριο χαρακτηριστικό του άσθματος. Ωστόσο η δική μας μελέτη είναι η πρώτη κλινική μελέτη παγκοσμίως που δείχνει βελτίωση της φλεγμονής αλλά και μείωση της λήψης φαρμάκων σε παιδιά με άσθμα».

Αυτή η πρώτη του είδους της λοιπόν κλινική μελέτη περιέλαβε 64 παιδιά ηλικίας 5-12 ετών από τη χώρα μας με ήπιο άσθμα (αυτό σημαίνει ότι τα παιδιά δεν λάμβαναν φάρμακα σε μόνιμη βάση αλλά μόνο όταν ήταν αναγκαίο εξαιτίας εμφάνισης συμπτωμάτων) τα οποία χωρίστηκαν σε δύο ομάδες. Οι ερευνητές έδωσαν στα μισά περίπου από αυτά οδηγίες για κατανάλωση δύο γευμάτων εβδομαδιαίως από λιπαρά ψάρια, και συγκεκριμένα από σαρδέλα ή γαύρο ή σκουμπρί ή κολιό ή πέστροφα ή σολομό (ως γεύμα ορίστηκαν τουλάχιστον 150 γραμμάρια ψαριού μετά το μαγείρεμά του) μέσα στο πλαίσιο του μεσογειακού προτύπου διατροφής επί έξι μήνες. Τα υπόλοιπα παιδιά ακολούθησαν τη συνήθη διατροφή τους επίσης επί ένα εξάμηνο.

Ιδού τι προέκυψε: στην ομάδα που κατανάλωνε λιπαρά ψάρια εμφανίστηκε στο τέλος του εξαμήνου μείωση της βρογχικής φλεγμονής κατά 14 μονάδες, ενώ την ίδια στιγμή στην ομάδα ελέγχου παρουσιάστηκε διατήρηση και σε κάποιες περιπτώσεις επιδείνωση της

φλεγμονής. Τι σημαίνουν αυτές οι 14 μονάδες; θα αναρωτιέστε – και δικαίως. Όπως εξηγεί η κυρία Παπαμιχαήλ, *«σύμφωνα με τις κατευθυντήριες οδηγίες της Αμερικανικής Εταιρείας Θώρακος οποιαδήποτε μείωση άνω των 10 μονάδων δείχνει σημαντική απόκριση στην αντιφλεγμονώδη θεραπεία»*. Με απλά λόγια, τα λιπαρά ψάρια φάνηκε να λειτουργούν θεραπευτικά στα παιδιά ή έστω να ενισχύουν το θεραπευτικό αποτέλεσμα των φαρμάκων τους – γεγονός που ενισχύεται από το εξίσου σημαντικό εύρημα της μελέτης ότι στην ομάδα τής... ψαροπαρέμβασης κατεγράφη μεγαλύτερη μείωση στη χρήση φαρμάκων σε σύγκριση με την ομάδα που δεν ακολούθησε την «ψαροδιατροφή».

Διατροφική ασπίδα προστασίας

Κατά την ερευνήτρια *«τα ευρήματα αυτά μαρτυρούν ότι είναι δυνατό να διαχειριστούμε τα συμπτώματα του άσθματος μέσω της υγιεινής διατροφής – δεν είναι μάλιστα τυχαίο ότι στοιχεία δείχνουν πως η ανθυγιεινή διατροφή που περιλαμβάνει πολλά επεξεργασμένα τρόφιμα και ζάχαρη ενισχύει τα ασθματικά συμπτώματα. Η ελληνική παραδοσιακή μεσογειακή διατροφή που είναι πλούσια σε φυτικά τρόφιμα και λιπαρά ψάρια θα μπορούσε να είναι ένας πολύ εύκολος, ασφαλής και αποτελεσματικός τρόπος για τη μείωση των συμπτωμάτων του παιδικού άσθματος. Τα λιπαρά ψάρια, όπως αυτά που χρησιμοποιήσαμε στη μελέτη,*

έχουν υψηλή περιεκτικότητα σε ω-3 λιπαρά οξέα τα οποία διαθέτουν αντιφλεγμονώδεις ιδιότητες. Δεν έχουν εξακριβωθεί ακόμη οι ακριβείς μηχανισμοί μέσω των οποίων τα ω-3 μειώνουν τη φλεγμονή των βρόγχων, εκτιμάται όμως ότι μεταξύ άλλων βάζουν φρένο σε προφλεγμονώδεις ουσίες που προάγουν το οίδημα των αεραγωγών, την έκκριση βλέννας, τη βρογχική φλεγμονή και τον βρογχόσπασμο».

Τα εντυπωσιακά – και κυρίως εύκολα στην εφαρμογή – καινούργια ευρήματα δεν συνιστούν αυτή τη στιγμή σύσταση προς τους γονείς των παιδιών με άσθμα. «Απαιτούνται μεγαλύτερες μελέτες ώστε να επιβεβαιωθεί η θετική αυτή επίδραση και η ομάδα μας διερευνά τα ενδεχόμενα τόσο της μελλοντικής διεξαγωγής μιας τέτοιας μεγαλύτερου εύρους μελέτης όσο και της επέκτασής της σε παιδιά με σοβαρό άσθμα ώστε να καταγραφούν τα πιθανά οφέλη από την κατανάλωση των λιπαρών ψαριών και στη συγκεκριμένη «δύσκολη ομάδα»» υπογραμμίζει η κυρία Παπαμιχαήλ. Προσθέτει ωστόσο ότι δεν πρέπει να ξεχνούμε ότι μιλούμε για μια διατροφική παρέμβαση που δεν συνδέεται με καμία παρενέργεια παρά μόνο με οφέλη για ολόκληρο τον οργανισμό, καθώς πλήθος μελετών έχουν δείξει πως τα ω-3 λιπαρά οξέα των ψαριών προσφέρουν προστασία ενάντια σε πολλές νόσους – από τα καρδιαγγειακά νοσήματα ως μορφές καρκίνου. Έτσι, μάλλον μόνο καλό μπορεί να κάνει το να τρώμε εμείς και τα παιδιά μας (με άσθμα και μη) λιπαρά ψάρια... στην υγεία του αναπνευστικού μας συστήματος (και όχι μόνο).

Άλλο τροφή, άλλο συμπλήρωμα!

Αφού ω-3 κυκλοφορούν ευρέως σε μορφή συμπληρωμάτων, γιατί να παιδευόμαστε με το μαγείρεμα ψαριών και να μην πάρουμε μια κάψουλα ώστε να αποκομίσουμε τα οφέλη; Ιδού η απάντηση διά στόματος της κυρίας Παπαμιχαήλ: «Έχουν υπάρξει μελέτες που διερευνούσαν τα πιθανά οφέλη από τη λήψη συμπληρωμάτων στο άσθμα. Ωστόσο τα ευρήματα ήταν αντικρουόμενα: κάποιες έδειχναν ως έναν βαθμό οφέλη, από άλλες όμως δεν προέκυπταν οφέλη ενάντια στα ασθματικά συμπτώματα. Ως φαίνεται λοιπόν δεν είναι μόνο τα ω-3 που κάνουν τη διαφορά, είναι η συνεργική δράση των ω-3 με τα υπόλοιπα θρεπτικά συστατικά που παρέχει η μεσογειακή διατροφή». Η ισχύς λοιπόν εν τη ενώσει ενάντια στη φλεγμονή των αεραγωγών που προκαλεί το άσθμα. Διότι μεσογειακή διατροφή σημαίνει και πολλά λαχανικά, χόρτα, φρούτα, μη επεξεργασμένα δημητριακά, όσπρια, ξηρούς καρπούς και ελαιόλαδο, μέτρια κατανάλωση γαλακτοκομικών και πουλερικών καθώς και λίγο ως πολύ λίγο κρέας και γλυκά (μαζί με ένα ποτηράκι κρασί σε κάθε γεύμα το οποίο ευφραίνει... σώμα και πνεύμα). Και αυτού του τύπου η διατροφή είναι πλούσια σε μονοακόρεστα λιπαρά οξέα καθώς και σε φυτικές ίνες και αντιοξειδωτικές ουσίες, όπως οι βιταμίνες E και C, η ρεσβερατρόλη, οι πολυφαινόλες, το σελήνιο και η γλουταθειόνη. Όλα αυτά σε συνεργασία με τα ω-3 λιπαρά οξέα των ψαριών – το εικοσαπεντανοϊκό οξύ (EPA) και το δοκοσαεξαενοϊκό οξύ (DHA) – φαίνεται ότι προσφέρουν έναν συνδυασμό ακαταμάχητο (εναντίον του άσθματος).

Additional Links (Greece):

1. BHMA Newspaper: https://www.tovima.gr/printed_post/sardela-kai-gayros-lfenantion-asthmatos/

2. Iatronet: <https://www.iatronet.gr/eidiseis-nea/epistimi-zwi/news/48324/erevna-i-katanalwsi-psariwn-voitha-sto-paidiko-asthma.html>

3. Zougla News: <https://www.zougla.gr/ygeia/article/eliniki-meleti-vrike-oti-ta-psaria-voi8oun-stin-katapolemissi-tou-as8matos>

Appendix 6A Online Supplement of Published Manuscripts

Manuscript 1: Maria M Papamichael, Catherine Itsiopoulos, Nugroho H Susanto and Bircan Erbas “Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? A systematic review of observational studies”. *Public Health Nutr* 20(15), 2722–2734.



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	3, 37-38
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4-6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I ²) for each meta-analysis.	-



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7-8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9-11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	12
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	-
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	-
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12-13
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14-15
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15-16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

Ref: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000.

Appendix 6A Online Supplement of Published Manuscripts

Manuscript 2

Maria Michelle Papamichael, Som Kumar Shrestha, Catherine Itsiopoulos, Bircan Erbas, 2018.
The role of fish intake on asthma in children: A meta-analysis of observational studies.
Pediatr Allergy Immunol. 2018; June 29:350–360. Epub 2018 March 5 doi: 10.1111/pai.12889.

Table S1. Characteristics of relevant studies in this systematic review

Author/Year Name of study/ Location	Study design	Sample size	Age	Age Endpoints Assessed	Dietary Exposure	Asthma outcome measured	Respiratory Assessment	Dietary Assessment	Exposure estimate/ Confounders	Findings	Study outcome
Hodge et al ³⁴ 1996 Australia	Case- control	584	8-11 y.o		All fish (Fresh fatty fish > 2% fat)	<ul style="list-style-type: none"> • Current asthma • Wheeze only • AHR 	Questionnaire	FFQ Categories: Daily, weekly, monthly, rarely or never. Fish categories: none, fresh fish, oily and non-oily.	Fatty fish intake \geq 1x/wk, Current asthma risk: <ul style="list-style-type: none"> • Fatty fish consumers: (ORadj: 0.26; 95%CI: 0.09-0.72; p<0.01) Fresh fish consumers: (ORadj: 0.52; 95% CI: 0.24-1.15; p>0.05) Non-fatty fish consumers (ORadj: 0.68; 95% CI: 0.3-1.54; p>0.05). Confounders: Sex, ethnicity, country of birth, atopy, respiratory infection in the first two years of life parental asthma, smoking	Lower prevalence of 'current asthma' 8.8% in fatty fish consumers, 15.6% non-fatty fish consumers and 23% never ate fish. Significant reduction in current asthma for children eating oily fish but not for wheeze only and AHR	✓
Nafstad et al ⁴⁰ 2003 Oslo Birth Cohort Study Norway	Cohort	2531	0-4 y.o	1, 2, 4 y.o	All fish	<ul style="list-style-type: none"> • Asthma risk 	Doctor diagnosed Questionnaire	Qualitative : Fish intake: Yes/No	<ul style="list-style-type: none"> • Asthma symptoms at 4 yrs: (ORadj:0.84; 95% CI=0.57-1.22, p=0.049) • Asthma risk in children with non- atopic parents: (ORadj 0.50; 95% CI: 0.30-0.83, p=0.031) • Asthma risk in children with atopic parents: (ORadj:1.00; 95%CI:0.61-1.63; p=0.093) Confounders: Parental atopy, atopic eczema at 0–6 months of age, gender, parity, birth weight,	Fish intake during first year of life ↓asthma symptoms at 4 yrs. Inverse relationship between fish intake and asthma risk in children with non-atopic parents	✓

maternal age at delivery, birth order, uterus-related pregnancy complications, pets at home, lower respiratory tract infections during first year of life, maternal education, family income, maternal smoking, breast-feeding duration												
Kull et al ¹¹ , 2006 BAMSE Study Sweden	Cohort	4,089	0-4 y.o	1, 2, 4 y.o	All fish	<ul style="list-style-type: none">• Asthma risk• Multiple allergic disease• Persistent allergic disease	Doctor-diagnosed	FFQ : Categories: Never, 1x/mo; 2-3x/mo; 1x/wk; ≥1x/wk	<p>• Early fish introduction at 3-8 mo, asthma risk at 4 y.o. (OR adj: 0.73, 95%CI: 0.55-0.97)</p> <p>Fish intake at 1 yr life (1x/mth):</p> <ul style="list-style-type: none">• Asthma risk at 4 years: (ORadj:0.94; 95%CI: 0 .57-1.56)• Allergy risk: (ORadj: 0.61; 95%CI: 0.45-0.84) <p>Fish intake at 1 yr life: (2-3x/mth)</p> <ul style="list-style-type: none">• Asthma risk at 4 yrs: (OR adj: 0.82; 95%CI: 0.51-1.29)• Allergy risk at 4 yrs: (OR adj: 0.58; 95%CI: 0.45-0.76) <p>Fish intake 1x/wk:</p> <ul style="list-style-type: none">• Asthma risk at 4 yrs: (OR adj: 0.66, 95%CI: 0.43-1.01)• Allergy risk OR adj: 0.50, 95%CI: 0.39-0.64) <p>Fish intake ≥1x/wk:</p> <ul style="list-style-type: none">• Asthma risk: (OR adj: 0.55; 95%CI: 0.34-0.87) p trend=0.003• Allergy risk at 4 yrs: (OR adj: 0.46; 95%CI: 0.35-0.6) p trend<0.001	Early fish introduction at 3-8 mo ↓ asthma risk in children at 4 y.o as compared to fish introduced later than 9 months.	Regular fish consumption (≥ 2/month) during 1 st year life ↓risk of asthma and allergy (eczema, rhinitis and sensitization) at 4 y.o. A dose-dependent reduced risk was observed for asthma (p trend <0.003) and all allergy (p trend <0.001)	✓

Oien et al ⁴² , 2010 Norway	Cohort	3,086	0-2 y.o	1 y.o, 2 y.o	All fish	<ul style="list-style-type: none"> • Parent-reported asthma • Doctor-diagnosed asthma 	Parent-report	FFQ Categories: Never, <1x/wk, 1x/wk, 2x/wk, 3x/wk, ≥4x/wk.	<p>Confounders: Parental allergic disease, maternal age, maternal smoking and breastfeeding.</p> <p>Any kind fish ≥ 1x/wk:</p> <ul style="list-style-type: none"> • Doctor-diagnosed asthma (OR: 0.81; 95% CI:0.6-1.09) p=0.16 • ≥ 1x/wk Oily fish • Doctor-diagnosed asthma (OR: 1.06; 95% CI:0.58-1.93) p=0.86 <p>≥ 1x/wk Lean fish</p> <ul style="list-style-type: none"> • Doctor-diagnosed asthma: (OR: 0.76; 95% CI:0.52-1.13) p=0.17 <p>Confounders: Gender, familial atopy, one parent & sibling, two parents & sibling, parental smoking 1 year after delivery, children's intake of cod liver oil, vegetables at 1 year of age, parental home-owner status, breast-feeding ≥ 4 months, pets.</p>	No significant association between fish intake and doctor-diagnosed asthma (p=0.16)	X
Willers et al ⁴³ , 2011 PIAMA Netherlands	Cohort	4,146	1-8 y.o	8 y.o	All fish	<ul style="list-style-type: none"> • Wheeze • Dyspnoea • Inhaled steroid use in previous 12 months • BHR • Asthma symptoms • Sensitization to inhaled allergens • Sensitization to food allergens 	ISAAC questionnaire BHR by medical exam	FFQ Categories: Never; < 1x/wk; 1-2 days/wk; 3-5 days/wk; 6-7 days/wk	<p>One consumption day/week increase in long-term intake of fish from 2-8 yrs and outcomes at 8 years:</p> <ul style="list-style-type: none"> • Wheeze (OR: 1.32; 95% CI: 0.97-1.80) • Inhaled steroid use (OR: 1.36; 95% CI: 0.98-1.88) • Dyspnoea (OR: 1.01; 95% CI: 0.75-1.35) • Asthma symptoms (ORadj:1.23; 95% CI:0.97-1.57) • BHR (OR 0.79; 95% CI: 0.58-1.09) 	<p>No statistically significant associations between early (2-3 years), late (7-8 years) or long-term fish intake on asthma or BHR at 8 y.o.</p> <p>However, early fish intake was inversely associated with BHR only.</p>	X

									Early fish intake: <ul style="list-style-type: none">• BHR (OR 0.76; 95% CI: 0.59–0.99)		
									Confounders: Sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at 8 yrs, breast feeding, presence of older siblings, birth weight, overweight mother, overweight child at 8 yrs geographical region		
Goksor et al ³⁹ , 2011 BAMSE study Sweden	Cohort	4,171	0-4.5 y.o	4.5 y.o	All fish	<ul style="list-style-type: none">• Recurrent wheeze• Multiple trigger wheeze• Episodic viral wheeze	ISAAC Questionnaire	FFQ: ≥ 1x/mo	Introduction fish < 9 months: <ul style="list-style-type: none">• Recurrent wheeze (ORadj: 0.6; 95% CI 0.4-0.8); p<0.05• Multiple trigger wheeze (ORadj: 0.6; 95% CI:0.3-0.99); p<0.05• Episodic viral wheeze (ORadj: 0.6; 95% CI:0.4-0.99); p<0.05 Confounders: Atopic hereditary, parental educational level, male, maternal smoking and medication during pregnancy, gestation age < 37 wks, caesarean section, antibiotics during 1 st year life, breast-feeding for ≥ 4 months, doctor-diagnosed food allergy during 1 st year life, eczema during first year of life, introduction of fish before 9 months, fish ≥ 1x/month at age 1 year.	Fish intake ≤ 9 mo ↓ wheezing risk at 4.5 y.o (P<0.05) for children treated with antibiotics during first week of life	✓
Kiefte-de-Jong et al ³⁷ , 2012	Cohort	7,210	12 mo, 14 mo	48 mo	All fish Fatty fish	<ul style="list-style-type: none">• Prevalence of wheezing	Physician diagnosed &	FFQ Categories:	No fish introduction during 1st year of life <ul style="list-style-type: none">• Wheezing risk at 48mo:	Fish introduced between 6- 12 months lower prevalence of	✓

Generation R Study Netherlands					> 10g fat per 100g fish); Lean fish: <10g fat per 100g fish; Serving size= 120g raw fish		ISAAC Questionnaire	No fish= <1/2 serving/ week; Eat fish: ≥ 1/2 serving/week	(ORadj: 1.57, 95%CI: 1.07-2.31; p=0.03) Introduction of fish 0-6 mo: • Wheezing risk at 48 mo (ORadj: 1.53, 95%CI: 1.07-2.19; p=0.03) Introduction of fish at 6-12 mo of age: • Wheezing risk at 48 mo (ORadj:0.64; 95% CI: 0.43-0.94, p=0.03) Confounders: Maternal age, maternal BMI, maternal alcohol, smoking during pregnancy, household income, maternal educational level, family history asthma, eczema, hay fever, allergy to house dust, maternal fish consumption during pregnancy, folic acid supplementation during pregnancy, parity, birth weight, gestational age, infant’s gender, infant’s ethnicity, breastfeeding duration, early day-care attendance, vitamin D supplementation in 1 st year life	wheezing in children at 48 months compared to ‘no’ fish introduced during 1 st year of life and fish introduced between 0-6 months of age (p=0.03)	
Magnusson et al ³⁶ , 2013 BAMSE Study Sweden	Cohort	3,285	1-12 y.o	1, 2, 4, 8, 12 y.o	All fish	• Prevalent asthma • Incidence asthma	Questionnaire	FFQ : Categories: Never, 1 x/mo; 2-3 x/mo;1x/wk; >1x/wk. Regular fish intake: ≥2-3 times/ mo ; Irregular: ≤ 1 time/mo	Fish intake at 1 year & prevalence asthma risk up to 12 y.o: Fish intake 1x/mth: • Prevalent asthma (ORadj:0.61; 95% CI:0.43-0.88, p≤0.001) Fish intake 2-3x/mth: • Prevalent asthma (ORadj:0.74; 95% CI:0.55-1.00, p≤0.001)	Regular fish intake at age 1 yr (≥2-3x/mo) ↓ prevalence and incidence of asthma up to 12 years. Reduced risk was dose-dependent for both outcomes (p<0.05)	✓

<p>Fish intake 1x/wk:</p> <ul style="list-style-type: none">• Prevalent asthma (ORadj:0.49; 95% CI:0.37-0.65, p≤0.001) <p>Fish intake >1x/wk:</p> <ul style="list-style-type: none">• Prevalent asthma (ORadj:0.54; 95% CI: 0.40-0.74, p≤0.001) <p>Regular fish intake ≥2-3x/mo at 1 year and prevalence asthma up to 12 y.o:</p> <ul style="list-style-type: none">• Prevalent asthma (ORadj: 0.71; 95% CI: 0.57-0.87; p=0.001) <ul style="list-style-type: none">• Overall Incidence asthma (ORadj: 0.80; 95% CI: 0.65-0.98; p=0.034) <p>Confounders: Sex, parental allergy, maternal smoking, SES status, breast- feeding duration, maternal age, overweight, parental smoking at age 8 yrs, child’s diet at age 8 yrs.</p>											
Nwaru et al ⁴¹ , 2013 DIPP Study Finland	Cohort	3,781	5 y.o	3 mo,6 mo, 12 mo; 5 y.o	All fish	<ul style="list-style-type: none">• All Asthma• Atopic asthma• Non-atopic asthma	ISAAC questionnaire	Questionnaire	Data not shown	Introduction of fish at 6-9 mo ↓ ‘All asthma’ (p<0.001) & ‘atopic asthma’ (p<0.05) at 5 yr	✓
Dotterund et al ³⁸ , 2013 PACT Study, Norway	Controlled Intervention Cohort	1,374	2 y.o	2 y.o	All fish Intervention 1.2g cod liver oil from 6-8 wks age and oily fish	<ul style="list-style-type: none">•Parent-reported asthma•Used asthma medication last 12 months•Wheeze	Questionnaire adapted from ISAAC	FFQ: Frequency: ≥1x/wk	<ul style="list-style-type: none">• Incidence of parent reported asthma: (ORc: 0.72 95% CI: 0.55-0.93; p=0.001)• Using asthma medication in last 12 months (ORc: 0.75; 95% CI: 0.58-0.96; p=0.02)• Wheeze	Intake of oily fish twice a week significantly lowered incidence in parental-reported doctor diagnosed asthma (p=0.01) and use of asthma medication (p=0.02)	✓

				(x2/wk) from 6 mo.					(ORc: 0.92; 95% CI: 0.8-1.05; p=0.23)		for children in the intervention group compared to the control group at age 2 years. Impact on asthma and medication use was significant in girls (p<0.01) not boys. No differences observed between the groups for the risk of wheeze (p=0.23)	
									After stratification by sex: <ul style="list-style-type: none">• Parent-reported asthma Girls: (ORc: 0.41; 95% CI: 0.24-0.70) p<0.01 Boys: (ORc:0.93; 95% CI: 0.68-1.26) <ul style="list-style-type: none">• Using asthma medication in last 12 months: Girls: (ORc: 0.46; 95% CI: 0.28-0.74) p<0.01 Boys: (ORc:0.95; 95% CI: 0.70-1.28)			
									Confounders: Birth weight, maternal atopy, respiratory infections, use of antibiotics, maternal healthcare centre, proportion of participants from each cohort in each public health centre			
Lumia et al ³⁵ , 2015 DIPP Study Finland	Nested case-control	182 asthma 728 control	0-6 y.o	3 mo, 6mo, 12 mo; 1-6 y.o	All Fish & fish products	<ul style="list-style-type: none">• All asthma• Atopic asthma• Non-atopic asthma	ISAAC Questionnaire	3-day food record	<ul style="list-style-type: none">• All asthma: (ORadj:0.87: 95% CI: 0.77-0.98; p=0.02)• Introduction of fish at 6.1-8 mo: All asthma: (ORadj:0.45: 95% CI: 0.28-0.72; p<0.05) Confounders: Gestational age, maternal age, maternal smoking during pregnancy, duration of breastfeeding, number of siblings, parental asthma or allergic rhinitis, birth weight,mode of	Early consumption of fish and fish products (6.1-8 mo) was associated with a decrease risk of all asthma in children at 6 years	✓	

Peat et al ³³ , 1992 Australia	Cross-sectional	4,366	7-9 y.o, 11-14 y.o	All fish	• BHR	Bronchial Challenge	FFQ Categories: Rarely, once/ month, once/wk, >1/wk	delivery, maternal vocational education, dogs at home during the first year of life, CMA, and age at introduction of solid foods (rye, wheat, barley, oats, fish, and egg) Children born outside of Australia: • BHR: (OR:0.56; 95% CI: 0.4-0.9; p<0.03) Regular fish meal (>1x/wk) • BHR: (OR:0.35; 95% CI: 0.1-0.9; p<0.04) Confounders: Parental asthma, atopy parental smoking, early respiratory illness, gender, born outside Australia, race, dwelling region	Children born outside of Australia had lower BHR than children born in Australia (8% v 17.6%; p<0.03). And eating fish regularly (>x1/week) had a significant protective effect against BHR compared to 'no' fish or eating fish (≤ 1x/wk) (p<0.04)	✓
Takemura et al ³² , 2002 Tokorozawa Childhood Asthma & Pollinosis Study Japan	Cross-sectional	23,782 (1,673 asthmatic 22,109 control)	6-15 y.o	All fish	• Current asthma	Questionnaire (Japan Environment Agency)	FFQ: Categories: None, 1-2/mth; 1-2x/wk; ≥3-4/wk	No fish: • Current asthma (ORadj:1.039; 95% CI:0.785-1.376) Fish intake 1-2x/wk: • Current asthma (ORadj:1.117;95% CI: 1.005-1.241) Fish intake: ≥3-4x/wk: • Current asthma (ORadj:1.319; 95% CI:0.896-1.943) Fish intake: 1-2x/mo • Current asthma Reference: (OR: 1.00) (p trend =0.0394)	Frequency of fish intake was positively associated with prevalence of asthma. Children that consumed fish 1-2x/wk had a significantly higher prevalence of asthma as compared to those who ate fish 1-2x/mo or no fish (p trend= 0.0394)	X

Antova et al ²⁹ , 2003 CESAR Study	Cross-sectional	20,271	7-11 y.o	All fish	<ul style="list-style-type: none"> • Winter cough • Persistent cough • Wheeze ever • Current wheeze 	ISAAC Questionnaire	FFQ: Categories: ≥1x/mo <1x/mo	<p>Confounders: Age, gender, parental history of asthma, fruit and vegetables</p> <p>Low fish intake (<1x/mo):</p> <ul style="list-style-type: none"> • Persistent cough (OR: 1.18, 95% CI: 1.04-1.34; p=0.01) • Wheeze ever: (OR: 1.14, 95% CI: 1.03-1.25; p=0.01) • Current wheeze: (OR: 1.21, 95% CI: 1.06-1.39; p=0.01) • Winter cough: (OR: 1.10; 95% CI: 0.99-1.23; p=0.07) <p>Confounders: Age, sex, area, presence of pets, presence of indoor moisture, use of gas oven for heating, additional unvented gas heating, number of smokers in household, mother's education, father's occupation, parent's allergy respondent, overcrowding (>1 person per room), all nutritional risk factors</p>	An increase in asthma symptoms was found for low fish intake (<1/month) and 'persistent cough' (p=0.01), 'wheeze ever' (p=0.01) and 'current wheeze' (p=0.01). But weakly associated with 'winter cough', (p=0.07).	✓
Farchi et al ²⁶ , 2003, SIDRIA Study, Italy	Cross-sectional	5,257	6-7 y.o	Blue fish	<ul style="list-style-type: none"> • Occurrence of wheeze in past 12 months • Shortness of breath with wheezing in past 12 months 	ISAAC Questionnaire	FFQ Categories: Never, < 1x/wk, 1-2 x/wk, 3-4x/wk, 5-7x/wk	<p>Fish intake: <1x/wk:</p> <ul style="list-style-type: none"> • Occurrence of wheeze OR: 0.66; 95% CI: 0.44-0.99) • Shortness of breath with wheeze (OR: 0.84; 95% CI: 0.52-1.35) <p>Fish intake: 1-2x/wk:</p> <ul style="list-style-type: none"> • Occurrence of wheeze (OR: 0.86; 95% CI: 0.01-1.23) • Shortness of breath with wheeze 	No correlation found between frequency of fish consumption and occurrence of wheeze (p= 0.343) or shortness of breath with wheeze (p=0.743) in children aged 6-7 years	X

								(OR:0.77; 95% CI: 0.5-1.19)		
								Fish intake: 3-4x/wk: <ul style="list-style-type: none"> • Occurrence of wheeze (OR: 0.77; 95% CI:0.32-1.86) • Shortness of breath with wheeze (OR:0.97; 95% CI:0.36-2.58) 		
								Confounders: Sex, study area, parental education, dampness, mould in the child's room, household crowding.		
Kim et al ²⁷ , 2005 Sweden	Cross-sectional	1,482	5-14 y.o	All fish	<ul style="list-style-type: none"> • Current asthma • Night time breathlessness • Doctor diagnosed asthma 	ISAAC Questionnaire	FFQ Categories: Never, <1x/wk, 1x/wk, >1x/wk, daily	Regular consumption of fish: <ul style="list-style-type: none"> • Night time breathlessness (OR:0.36; 95% CI:0.17-0.78) p<0.05 	Children consuming fish regularly had less night time breathlessness (p<0.05) doctor-diagnosed asthma (p<0.01) and current asthma (p<0.01) even after excluding children with food allergy or intolerance (p<0.01)	✓
								Doctor-diagnosed asthma (OR:0.54; 95% CI: 0.35-0.84) p<0.01 <ul style="list-style-type: none"> • Current asthma (OR:0.51; 95% CI: 0.31-0.84) p<0.01 		
								Children with no food allergy/food intolerance: <ul style="list-style-type: none"> • Doctor-diagnosed asthma (OR: 0.44;95% CI:0.25-0.76) p<0.01 • Current asthma (OR:0.37; 95% CI: 0.19-0.72) p<0.01 		
								Confounders: Age, gender, type of fat and dietary factors		
Tabak et al ³¹ , 2006 Netherlands	Cross-sectional	598	8-13 y.o	All fish	<ul style="list-style-type: none"> • Current wheeze • Current asthma • Atopic wheeze with BHR • Atopic asthma with BHR 	ISAAC Questionnaire	FFQ Categories: times per year/month/week/day/never; Always, mostly /often/sometimes/seldom/never	<ul style="list-style-type: none"> • Current wheeze (OR:0.44; 95% CI: 0.21-0.93) P<0.01) • Current asthma (OR:0.34, 95% CI:0.13-0.85; p<0.01) • Atopic asthma with BHR: (OR:0.12; 95% CI: 0.02-0.66) • Atopic wheeze with BHR: 	High fish intake (15g/d) was inversely associated with current wheeze (p<0.01), current asthma (p<0.01), atopic wheeze and atopic asthma in children	✓

(OR:0.15; 95%CI: 0.03-0.63)										
Confounders: Maternal educational level, foreign descent, total energy intake.										
Chatzi et al ¹⁰ , 2007 Spain	Cross-sectional	460	6.5 y.o	All fish	<ul style="list-style-type: none">• Current wheeze• Atopic wheeze• Atopy	Questionnaire	FFQ Categories: Never, <1x/mo- >6x/d	High intake of fish (60.5g/d) and prevalence of: <ul style="list-style-type: none">• Current wheeze: (ORadj:0.48, 95%CI:0.21-1.09; p=0.075)• Atopic wheeze: (ORadj:0.56, 95%CI: 0.16-2.00; p=0.338)• Atopy: (ORadj:0.43, 95%CI: 0.21-0.90; p=0.033) Confounders: Gender, maternal and paternal asthma and atopy, maternal smoking, BMI at age 6.5 yrs, parental education, social class, breast-feeding, fish intake during pregnancy, number of siblings at age 6.5 yr, energy intake.	High intake of fish (60.5g/d) was inversely associated with prevalence of atopy and not current or atopic wheeze.	X
Rodriguez et al ²⁵ , 2010 Spain	Cross-sectional	638	8-13 y.o	All fish	<ul style="list-style-type: none">• Current asthma	Questionnaire	3-day dietary record	Fish consumption (59.8g/d) <ul style="list-style-type: none">• Current asthma: (ORadj:1.22, 95%CI: 0.38-3.98 p=0.162) Confounders: Energy intake, fat intake, age, gender, BMI, parental asthma, atopic eczema, allergic rhinitis, parental cigarette smoking	No association between fish intake (59.8g/d) and asthma symptoms in children.	X
Nagel et al ²⁴ , 2010 ISAAC International Study	Cross-sectional	50,004	8-12 y.o	All fish	<ul style="list-style-type: none">• Asthma ever• Wheeze in past year• SPT• BHR	ISSAC Questionnaire	FFQ Categories: <1x/wk, >1x/wk, >1x/d	All children and fish intake ≥3x/wk: <ul style="list-style-type: none">• Asthma ever (OR adj: 0.92, 95%CI: 0.78-1.08; p trend= 0.04)	High intake of fish (≥3x/wk) as compared to never or occasionally was associated with lower	✓

• **Wheeze past year**
(OR adj:0.87; 95%CI: 0.74-1.03;
p trend= 0.29)

All children and consumption of fish (>1-2x/wk) in affluent countries:

• **Wheeze in past year**
(ORadj:0.85; 95%CI:0.74-0.97)

All children and consumption of fish (>1-2x/wk) in non- affluent countries:

• **Wheeze in past year**
(ORadj: 0.91; 95%CI:0.75-1.10)
p=0.589

Children SPT positive in non-affluent countries and fish consumed (>1-2/week):

• **Wheeze in past year SPT positive**
(OR adj: 0.51; 95%CI: 0.32-0.81)
p=0.001

Children SPT positive in affluent countries and fish consumed(>1-2/week):

• **Wheeze in past year SPT positive**
(ORadj: 1.02; 95%CI:0.76-1.35)
p=0.153

• **BHR**
OR adj: 1.34; 95%CI: 0.83-2.15;
p trend= 0.30)

Confounders: Sex, age, exposure to tobacco smoke, no. of siblings, parental atopy, exercise, maternal education

prevalence of asthma (p=0.04). And regular consumption of fish (>1-2/week) resulted in lower prevalence of wheeze during the previous year for all children in affluent countries and in children with positive SPT in non-affluent countries

Kunitsugu et al ²⁸ , 2012 Shunan Child Health (SCH) Study Japan	Cross-sectional	410 (138 asthma, 137 control, 135 eczema)	10-11 13-14 y.o	All fish & seafood	• Asthma	ISAAC Questionnaire	Self-reported Dietary History Questionnaire	<p>All fish intake: • Asthma (OR: 0.34; 95% CI: 0.14-0.81; p trend= 0.083)</p> <p>Fatty fish/dried fish intake: • Asthma (OR: 0.42; 95% CI: 0.18-0.96 p trend=0.374)</p> <p>Seafood intake : • Asthma (OR:0.34; 95% CI: 0.15-0.79; p p trend= 0.067)</p> <p>Confounders: Sex, school grade, parental smoking, single parent, siblings, school, residential area</p>	No statistically significant association was found between 'all fish' intake (p trend= 0.083), 'fatty/ dried fish' (p trend =0.374), or seafood and asthma (p trend= 0.067)	X
Saadeh et al ²³ , 2015, ISAAC Study, France	Cross-sectional	7,432	9-11 y.o	White fish	<ul style="list-style-type: none"> • Past year wheeze • Atopic wheeze • Lifetime asthma • SPT positive • BHR 	ISAAC Questionnaire	FFQ Frequencies: Never/occasionally; 1-2x/wk, ≥3x/wk	<p>Consumption of white fish (1-2x/wk): • Past year wheeze: (ORadj:0.75; 95% CI: 0.53-0.93; p trend= 0.028)</p> <p>• Atopic wheeze (ORadj:2.78; 95% CI:0.66-11.61); p trend= 0.042</p> <p>• SPT positive (ORadj:1.05; 95% CI: 0.69-1.61; p trend= 0.882)</p> <p>• Life-time asthma (ORadj:1.11; 95% CI: 0.61-2.03; p=0.261)</p> <p>• BHR (ORadj:1.44; 95% CI: 0.69-3.02 p trend= 0.524)</p> <p>Fish intake in SPT negative • Past year wheeze</p>	White fish intake (1-2x/week) was associated with lower prevalence of wheezing in children (p=0.028), but not for atopic wheeze, life-time asthma or BHR. After stratification for SPT positive, fish intake was correlated significantly to less wheezing in non-atopic children (p= 0.04).	✓

								(ORadj:0.61; 95%CI: 0.43-0.87; p = 0.04)		
								Confounders: Gender, place of residence, parental atopic disease number of siblings, maternal education, parental ethnic origins, breast-feeding, day care centre or nursery, overweight, obesity and current exposure to environmental tobacco smoke		
Xu et al ³⁰ , 2016 China	Cross-sectional	13,877	0-14 y.o	All fish & seafood	• Prevalence asthma	ISAAC Questionnaire NEAAC Questionnaire	Questionnaire	-	In 14% of children fish and shrimp consumption ↑ triggered asthma symptoms especially in children ≥ 6 years (p<0.05)	X

Key: mo= Months; yr(s)=Year(s); wk(s)=Week(s); y.o=Years old; All fish = Lean & fatty fish; AHR: Airway Hyperresponsiveness ; BHR= Bronchial Hyperresponsiveness; SPT= Skin Prick Test for atopy; ≥ x1/wk = More than once a week; ORc= Odds Ratio crude; ORadj= Odds Ratio after adjusting for confounding factors, - = No data provided in study; ↓ = Reduction; ↑ = Increase; X= No effect or adverse effect on asthma symptoms; √= Improvement in asthma symptoms.

Exposure- 'All fish'; Outcome- 'current asthma'

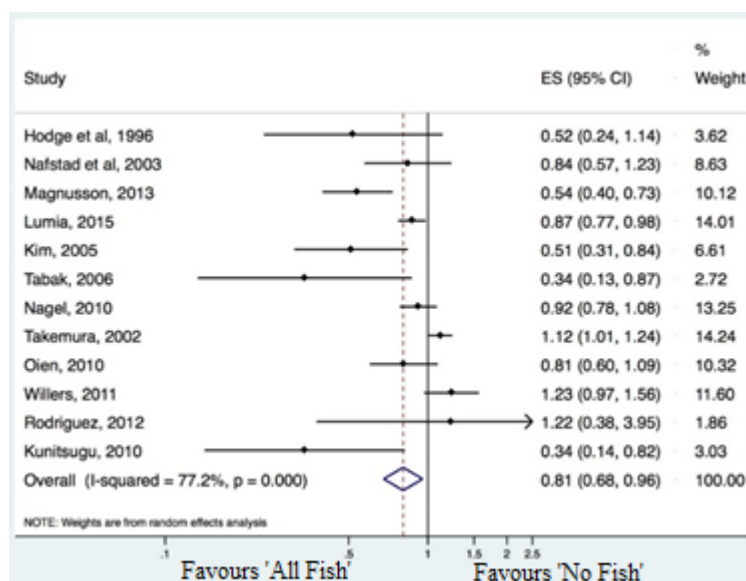


Fig S1. Forest plot of 'all study designs' and 'All Fish' intake versus 'No Fish' for 'current asthma' in children aged 2-15 y.o.

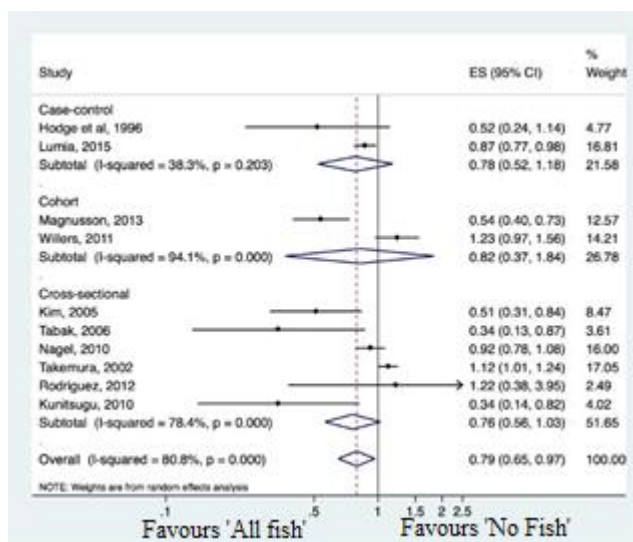


Fig S2. Forest plot of case-control trials in children (6-11 years), cohorts (8-12 years) and cross-sectional studies (5-15 years) for 'All Fish' intake versus 'No fish' and 'current asthma'

Exposure- 'All fish'; Outcome- 'current wheeze'

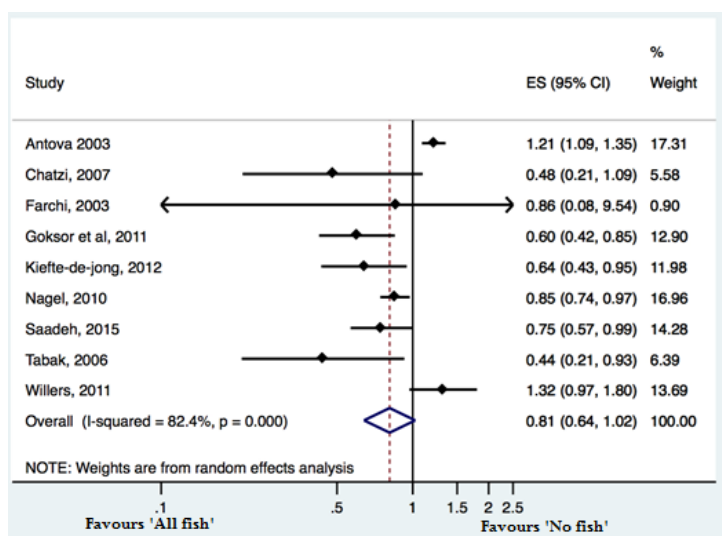


Fig S3. Forest plot of all study designs combined, children (0-13 years old) for 'All Fish' intake versus 'No Fish' and outcome 'current wheeze'.

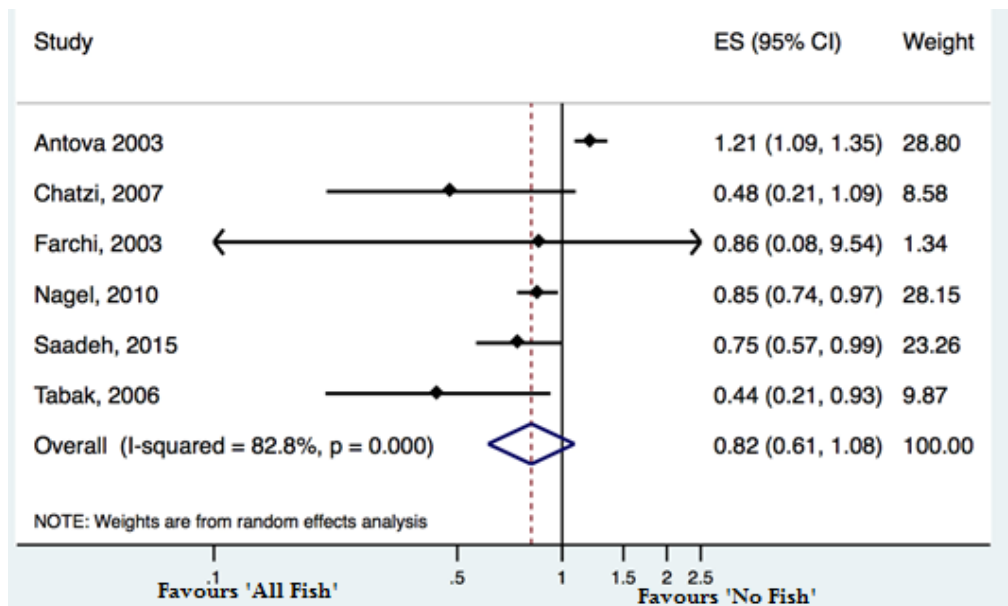


Fig S4. Forest plot of cross-sectional studies including children (6-13 years old) for 'All Fish' intake versus 'No Fish' and outcome 'current wheeze'.

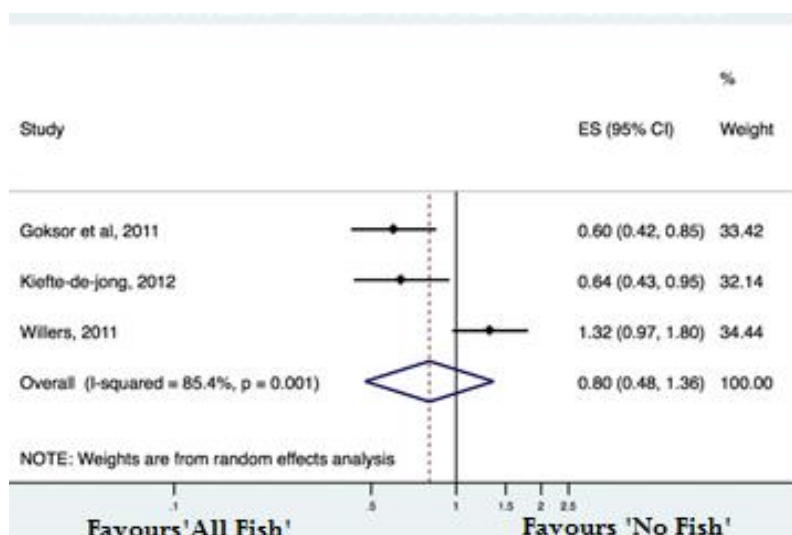


Fig S5_ Forest plot of cohort studies children (0-8 years old) for 'All' Fish intake versus 'No Fish' and outcome 'current wheeze'.

Exposure-'Fatty fish'; Outcome-'current asthma'

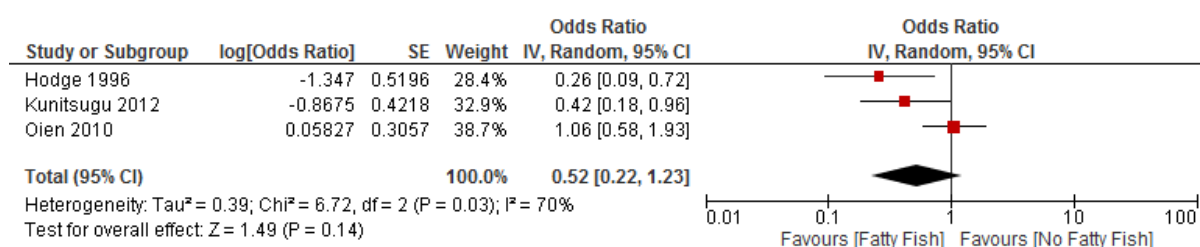


Fig S6. Forest plot comparing 'Fatty' Fish intake versus 'No Fatty' Fish and 'current asthma' in children 2-14 years old in the combined analysis.

Supplement

Box S1. Details of PubMed search strategies

Search	Query	Items found
S3 (S1 OR S2)	Search (fish or fatty fish or oily fish or omega 3 fatty acids or n-3 long chain polyunsaturated fatty acids) AND asthma AND children) OR ((Fish or fatty fish or oily fish or omega 3 fatty acids or n-3 long chain polyunsaturated fatty acids) AND childhood asthma)	333
S2	Search (Fish or fatty fish or oily fish or omega 3 fatty acids or n-3 long chain polyunsaturated fatty acids) AND childhood asthma	116
S1	Search (Fish or fatty fish or oily fish or omega 3 fatty acids or n-3 long chain polyunsaturated fatty acids AND asthma) AND children	326

Search retrieved from <http://www.ncbi.nlm.nih.gov>. Accessed on 5.7.17.

Supplement Data S2

Quality Assessment Tool for Systematic review Fish intake & Paediatric Asthma according to Zaza et al (2000)

Reviewer name: _____ Date: _____

Paper title: _____

First author: _____

Year Published _____ Journal _____

#	Question	Responses	Notes/Answer/Score
1 (a)	Where was the study done?		
1 (b)	Population density	<ul style="list-style-type: none"> • Urban • Suburban • Rural • Mixed • Not reported 	
2	How were outcome and other independent (or predictor) variables measured?	<ul style="list-style-type: none"> • Interview • Self-administered questionnaire • Hospital records • Record review • Observation • Not reported/did not assess 	
3	Over what time period (include dates) and at what intervals were outcomes and other variables measured?		
4	Indicate the study design	(1) Expert opinion/case study (2) Observational study without control group (3) Controlled observational study (4) Case control study (5) Cohort study (6) Quasi experimental study (7) Experimental study Other, specify (0) Can't tell	
5	Was the population well described?	Yes (1) No (0) N/A	
6	Were the eligibility criteria required to enter the study population well described?	Yes (1) No (0) N/A	

#	Question	Responses	Notes/Answer/Score
7	Did the authors specify the sampling frame or universe of selection for the study population?	Yes (1) No (0) N/A	
8	Was the population that served as the unit of analysis the entire eligible population or a probability sample at the point of observation?	Yes (1) No (0) N/A	
9	Was there attempt to measure exposure?	Yes (1) No (0) N/A	
10	Were the exposure measures valid measures?	Yes (1) No (0) N/A	
11	Were the exposure measures reliable?	Yes (1) No (0) N/A	
12	Were the outcome and other independent (or predictor) variables valid measures of the outcome of interest?	Yes (1) No (0) N/A	
13	Were the outcome and other independent (or predictor) variables reliable (consistent and reproducible) measures of the outcome of interest?	Yes (1) No (0) N/A	
14	Did the authors conduct appropriate analysis by conducting statistical testing?	Yes (1) No (0) N/A	
15	Did the authors report which statistical tests were used?	Yes (1) No (0) N/A	
16	Were the statistical tests appropriate for the study design?	Yes (1) No (0) N/A	
17	Was there controlling for design effects in the statistical model?	Yes (1) No (0) N/A	
18	Was there controlling for repeated measures in the analysis, for study designs in which the same population was followed with repeated measurements over time?	Yes (1) No (0) N/A	
19	Was there accounting for different levels of exposure in segments of the study population in the analysis?	Yes (1) No (0) N/A Can't tell (0)	
20	If the authors analysed group-level and individual-level covariates in the same statistical model, was the model designed to handle multi-level data?	Yes (1) No (0) N/A Can't tell (0)	

#	Question	Responses	Notes/Answer/Score
21	Were there other problems with data analysis that limit interpretation of the results of the study? If yes, describe	No (1) Yes (0) N/A Can't tell (0)	
22	Considering study design, were appropriate methods for controlling confounding variables and limiting potential biases used?	Yes (1) No (0) N/A Can't tell (0)	
23	Did the authors identify and discuss potential biases or unmeasured/contextual confounders that may account for or influence the observed results and explicitly state how they assessed these potential confounders and biases? Describe these factors and, if possible, comment on the likely direction of bias.	Yes (1) No (0) N/A Can't tell (0)	
24	Are there are additional biases not covered in other categories that the authors did not address, if yes, list these as well.	Yes (0) No (1) N/A Can't tell (0)	

Ref: Zaza, S., Wright-De Agüero, L. K., Briss, P. A., Truman, B. I., Hopkins, D. P., Hennessy, M. H., . . . Teutsch, S. M. (2000). Data collection instrument and procedure for systematic reviews in the Guide to Community Preventive Services. *American journal of preventive medicine*, 18(1), 44-74.

Supplement Data S4



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4-5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-6, Supp 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8-9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources), any assumptions, simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8, 22; Supp 2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8-9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	9; Supp 2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9-12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	13-15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	28-42
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	15-18; Supp 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	13-15
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18-22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	22

Ref: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.

Appendix 6A Online Supplement of Published Manuscripts

Manuscript 4

Papamichael MM; Katsardis Ch; Tsoukalas D; Erbas B; Itsiopoulos C (2019). Weight status and Respiratory Health in Asthmatic Children. *Lung* (2019) 197:777-782

DOI 10.1007/s00408-019-00273-w

ONLINE RESOURCE 1 (ESM_1)

METHODS

Study design

The Mediterranean diet intervention study was a two-arm randomized controlled trial (RCT) of six-months duration examining the effect of the Traditional Greek Mediterranean diet enriched with fatty fish on asthma symptoms and lung function in Greek school-children suffering with asthma. The primary outcome was based on spirometry parameter FEV₁.

Subjects

Seventy-two (72) children, 54% boys, 46% girls were voluntarily enrolled from a single-centre pediatric asthma clinic in the greater city of Athens, Greece during November 1st to December 31st, 2016.

Inclusion criteria were children suffering with physician-diagnosed mild asthma, 5-12 years old and willing to consume fatty fish. Subjects were excluded if they had severe or chronic asthma, gastroesophageal reflux disease, cystic fibrosis, congenital respiratory disease, food allergies, taking multiple glucocorticoids, high-dose multivitamins, fish oil supplements, being vegetarian and not willing to modify their diet.

Mild asthma was defined as stated by the Global Initiative for Asthma (GINA) guidelines which include day time symptoms and the need for reliever medication less than twice weekly, no night-waking symptoms or limitations in daily activities due to asthma and FEV₁ > 80% predicted [1].

After parents signed written informed consent, eligible subjects were randomized by the physician equally to intervention versus control groups with a 1:1 allocation ratio using an internet platform (<http://www.randomization.com>).

Intervention

The intervention group was instructed to consume two fatty fish meals per week (at least 150 g of cooked filleted fish per meal) as part of the Traditional Greek Mediterranean diet for a period of 6 months. Fatty fish included were sardines, trout, salmon, mackerel, anchovies, fresh, frozen and farmed. In contrast, the control group consumed their habitual diet.

Both groups were provided by the dietician with guidelines on healthy eating which correlates to the Traditional Greek Mediterranean dietary pattern as stated by the Hellenic Ministry of Health and Welfare (1999)[2]. During the study period all subjects were monitored by the dietician fortnightly via telephone, text, e-mails and face-to-face consultation.

Assessments

Subjects were assessed at baseline and at the end of six months during medical consultations.

Anthropometry

All subjects had their weights and heights measured according to a standard protocol[3].

After subjects removed shoes and heavy clothing body weight was measured to the nearest 0.1 kg on calibrated electronic scales (Seca Corp.). Then subjects were positioned in the standard Frankfurt horizontal plane and standing height was measured to the nearest 0.1 cm using a stadiometer (Seca Corp., Hanover, MD, USA). Body mass index (BMI) was calculated (kg/m^2) and study participants were classified as normal weight, overweight and obese using the Hellenic Paediatric Growth Charts [4].

Evaluation of Lung function

Subjects withheld from short-acting bronchodilators at least four hours before the procedure. For the purpose of this study spirometry and FeNO values pre-bronchodilator administration were used in statistical analyses.

Spirometry

Pulmonary function tests were performed at the pediatric asthma clinic by trained professionals, in a quiet environment at room temperature, using a portable spirometer (MIR Spirobank II; MIR Inc., New Orleans, LA, USA) in accordance to the guidelines published by the American Thoracic Society (ATS)/European Respiratory Society (ERS) protocol [5]. Spirometry was undertaken in the standing position with a nose-clip. The mouth-piece was placed into the participant's mouth with lips sealed firmly around the mouth-piece. The participant was instructed to inhale to total lung capacity and to exhale as hard and as fast as possible without a pause and then a deep breathe was inhaled to total lung capacity. The best spirometry value of

three acceptable and reproducible maximal flow-volume curves was recorded. Normal pulmonary function was considered values of forced expiratory volume in 1 s (FEV₁) greater than 80% predicted and variation in FEV₁ of 10–12% to be clinically significant in children [6]. Spirometry was repeated 15 minutes after 4 inhalations of bronchodilator delivered through a spacer.

FeNO

Fractional exhaled nitric oxide (FeNO) was measured using a FeNO analyser (NO Breath, Benfont Inc., UK) with a fixed flow rate of 0.05 L/s and exhalation duration of 6 seconds in accordance with ATS/ERS guidelines [7]. No bronchial inflammation was denoted by FeNO values < 20 ppb [8] .

Questionnaires

Given that subjects were younger than 12 years old, parents were used as surrogates to complete questionnaires. Details on medical history and adherence to the Mediterranean diet were collected via telephone interviews that were conducted by the dietician during the same week of medical examinations.

Evaluation of socio-demographic characteristics

A composite questionnaire was developed for the purposes of this study to retrieve socio-demographic information, asthma control, quality of life, dietary habits and medical history.

Socio-demographics included information relating to residential area, postcode, parents' ethnicity, race, marital status, employment, education level, family income, number of siblings, birth rank of participant and type of school attended.

Asthma control

A Greek translation of the validated Asthma Control Questionnaire (ACQ) was used to assess the degree of asthma control in children and adolescents 6-16 years old [9]. This questionnaire consists of 7 items that assess the presence of symptoms, night-time waking, activity limitation, shortness of breath, wheeze, and rescue medication use during the past 7 days on a 7-point scale (0= totally controlled to 6= extremely poorly controlled) including pulmonary function % FEV₁ predicted. The overall ACQ score is the mean of the 7 items. A score < 0.75 is defined as 'well-controlled' asthma and ≥ 1.5 as 'extremely poorly controlled'.

Asthma-related quality of life

Asthma-related quality of life in pediatric patients was assessed using the Greek translation of the 13-item Mini Paediatric Asthma Quality of Life Questionnaire (Mini PAQLQ) [10,11]. This

questionnaire measures physical, emotional and social problems that are experienced by asthma children. Patients are asked to recall their experiences during the previous 7 days and respond to questions on a 7-point scale (7=no impairment, 1= severe impairment). The final score is the mean of the 13 responses.

Medical history and physical activity

Medical history and data on parental smoking status, parental allergy during childhood and adulthood, gestation details (pregnancy duration, child birth weight, mode of delivery), breast-feeding duration, participant's age of asthma onset and asthma-related allergies (rhinitis, eczema, conjunctivitis), food allergy as well as vitamin intake and asthma medication use were retrieved.

Physical activity status was evaluated as defined in the validated International Study on Asthma and Allergies (ISAAC) Phase 3 Environmental Questionnaire [12]. Regular physical activity was considered more than or equal to three times per week.

Participation in physical activity was assessed by the following question:

“How many times per week does your child engage in vigorous exercise?” Possible responses were never/rarely, 1-2 times/week and more than 3 times/week.

Dietary Evaluation

Dietary evaluation was measured using a 27- item Food Frequency Questionnaire (FFQ) that was based on the validated semi-quantitative PANACEA-FFQ for Greek children aged 10–12 years [13]. Information about the frequency of consumption of foods and beverages on a daily and weekly basis usually consumed in Greece as well as dietary behaviours (*i.e.*, eating breakfast, frequency of fast food consumption and use of olive oil in cooking) were recorded. In particular, the frequency of consumption of dairy products, fruit, vegetables/salads, legumes, breakfast cereals, bread, pasta, rice, red/white meat, seafood, fatty/lean fish, margarine, nuts, olive oil, fast food (hamburger, pizza, souvlaki), pies, sweets, salty snacks (such as potato chips), sodas, sport drinks and Traditional Greek meals such as mousaka, stuffed tomatoes with rice and pastitsio. Typical serving sizes of the aforementioned food and beverages were standard units for measurements. The FFQ was completed by parents during medical consultations in the presence of the dietician and staff.

In order to validate data in FFQs, three 24-hr food recalls were dietician-administered during telephone interviews at baseline, three and six-months.

Adherence to the Mediterranean diet

Adherence to the Mediterranean diet was evaluated using the 16-item KIDMED questionnaire which is a Mediterranean Diet Quality Index specifically designed for Spanish children and adolescents [14]. The index is derived from the 16 components that summarize the characteristics of the Mediterranean dietary pattern. Possible responses to each question is either Yes or No. Twelve questions denote a positive connotation with respect to the Mediterranean diet and are assigned a value of +1; 4 questions denote a negative connotation and are assigned a value of -1. The total score ranges from 0 to 12 where ≥ 8 denotes optimal adherence to the Mediterranean dietary pattern, 4-7 improvement need and ≤ 3 very low diet quality. Higher values of the score indicate high adherence to this dietary pattern. More specifically, the KIDMED index presumes a daily consumption of at least one serving of fruit and vegetables; at least three servings of dairy products daily (one dairy product for breakfast and at least two servings of yogurt and/or cheese during the rest of the day); consumption of grains and cereals is recommended daily for breakfast; pasta or rice should be consumed at least five times per week; at least 2–3 servings of nuts and fish per week; as well as two servings of pulses weekly. Olive oil is recommended for culinary use, but no frequency is suggested. Dietary behaviours that are viewed as detrimental to the principles of the Mediterranean diet include frequent intake of sweets, candies, commercially baked goods and pastries for breakfast, consumption of fast-foods, and breakfast skipping.

Nutritional biomarkers

Fatty acid composition (Omega 3 and Omega 6 fatty acids, EPA, DHA), organic acids and vitamin D status of subjects were assessed from urinary and blood samples that were collected, prepared, analysed and stored at a private metabolomics clinic in Athens, Greece.

Bioethics

All procedures performed in this study were in accordance with the ethical standards of the institution (La Trobe University Human Ethics Committee, HEC 16-035) and with the 1964 Helsinki declaration. The study protocol was registered with the Australian and New Zealand Clinical Trial Registry (www.ANZCTR.org.au/ACTRN12616000492459p).

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11. Wing A, Upton J, Svensson K, Weller P, Fletcher M, Walker S (2012) The standardized and mini versions of the PAQLQ are valid, reliable, and responsive measurement tools. *J Clin Epidemiol* 65 (6):643-650. doi:10.1016/j.jclinepi.2011.12.009

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<https://www.ncbi.nlm.nih.gov/pubmed/15482620>

Appendix 6A Online Supplement of Published Manuscripts

Manuscript 5 : M.M .Papamichael Ch. Katsardis, K. Lambert, D. Tsoukalas, M. Koutsilieris, B. Erbas & C. Itsiopoulos, 2019. Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial. *J Hum Nutr Diet* 32(2): 185-197.

Supplement

Appendix S1

Biochemical tests

Patients were requested to abstain from fluid and food consumption at least two hours after the last meal before testing. Venous samples (4ml) were collected from children following a 2 hour fast. The samples were centrifuged and plasma decanted from the supernatant and were stored at -20°C until analysis, within 24 h to avoid degradation. In case of hemolysis blood collection was repeated. The internal standard mixture (200 µL methyl nonadecanoate in hexane containing BHT) was added to 100 mL plasma. Fatty acid hydrolysis and derivatization into methyl esters was performed by adding 5% v/v Methanolic HCl. Transmethylation was performed at 90°C for 60 min. The samples were then brought to room temperature and extraction of FA methyl esters were performed using hexane. They were transferred to GC injection vials with a crimp cap. Mass spectrometry allows direct detection and identification of fatty acids in plasma without affecting quantity or quality, thus lipid extraction before methylation was not included {Stellaard, 1990 #439}.

Gas chromatography/mass spectrometry

The carrier gas used was helium and the sample injection volume was 1 µL. Analysis was performed on an Agilent 6890/5975C GC-MS operating in electron ionization mode. For the separation of Fatty acid methyl esters an HP-5 ms capillary column (30 m x 250 µm x 0.25 µm) was used. The initial oven temperature was 70°C, the ramp rate was 4°C/min, and the final temperature was 290°C, held for 4 min. Acquisition was in the scan mode.

Chemicals

Methyl nonadecanoate (74208, Fluka) was used as an internal standard. A mixture of Fatty acid methyl esters (47885-U, Supelco) was used for calibration of the standard mixture. All other solvents used were of the highest purity available (methanol (Merck), n-hexane (Merck), HCL (301721, Sigma-Aldrich), 2,6-di-tert-butyl-4-methylphenol (BHT, B1378, Aldrich)).

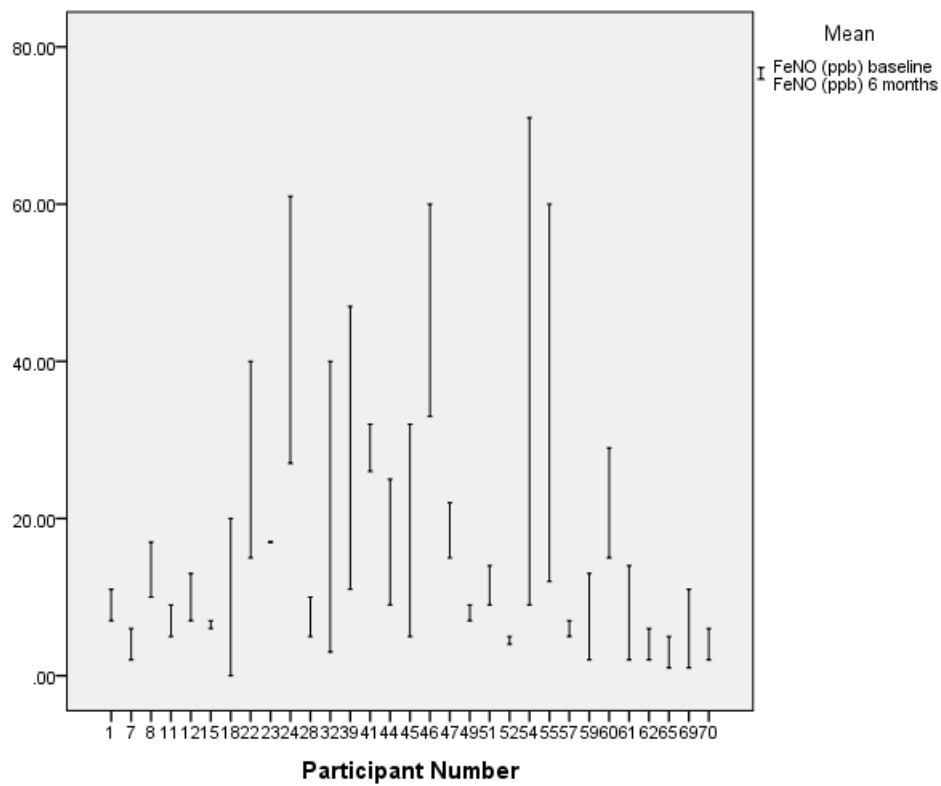
Supplement

Figure S1. FeNO values from baseline to six months for participants in the intervention group

Figure S1 depicts that at six months there was a decreasing trend in FeNO values for participants in the intervention group, which indicates a reduction in bronchial inflammation

Appendix 6A Online Supplement of Published Manuscripts

Manuscript 6: Maria Michelle Papamichael, Charis Katsardis, Bircan Erbas, Catherine Itsiopoulos, Dimitris Tsoukalas, 2019. *Urinary organic acids as biomarkers in the assessment of pulmonary function in children with asthma. J Nutrition Research 2019; 61: 31-40.*

NUTRITION RESEARCH 61 (2019) 31–40

Supplemental Table S1

Table S1. Percentiles of urinary organic acid levels for total sample of children.

Urine OAs (mmol/mol Crea)	Percentile 05 th	Percentile 10 th	Percentile 25 th	Percentile 50 th	Percentile 75 th	Percentile 95 th
Citric	31.50	34.95	66.65	87.40	149.10	282.20
Aconitic	22.30	25.02	31.75	40.65	52.40	72.70
Isocitric	3.70	4.25	5.50	6.90	8.45	10.90
2 -Ketoglutaric	8.40	11.00	16.65	23.35	31.45	49.10
Succinic	1.10	1.20	1.70	3.40	5.60	16.60
Fumaric	1.00	1.00	1.00	1.20	1.30	1.30
Malic	0.90	0.95	1.00	1.00	1.20	2.30
3-Hydroxy 3-methylglutaric	1.00	1.42	2.20	3.50	6.40	14.30
Lactic	2.40	2.70	3.70	4.70	6.40	13.00
Pyruvate	3.60	4.46	5.60	7.10	9.90	13.70
3-Hydroxybutyric	1.10	1.10	1.20	1.60	2.20	274.70
Pyroglutamic	5.60	6.26	10.10	15.90	20.50	27.10
2- Ketoisocaproic	1.30	1.30	1.30	1.30	1.30	1.30
2-Keto 3-methylvaleric	1.20	1.20	1.20	2.00	2.10	2.10
3-Hydroxyisovaleric	4.00	6.28	12.00	17.70	24.80	46.60
Methylmalonic	1.00	1.07	1.10	1.30	1.70	2.70
Homovanillic	1.30	1.40	2.35	3.00	4.30	6.10
5-Hydroxy-indole-acetic	1.00	1.14	1.80	3.00	3.90	7.40
Vanillilmandelic	1.10	1.26	1.70	2.10	2.70	3.70
4-Hydroxyphenylacetic	5.60	6.20	9.50	12.40	17.30	29.70
Orotic	1.10	1.10	1.10	1.10	1.10	1.10
Glutaric	0.90	0.90	1.00	1.20	1.45	1.60
2-Hydroxyglutaric	1.50	1.64	2.10	2.90	3.80	5.10
Glycolic	13.30	14.95	20.15	29.55	41.45	66.00
Oxalic	1.70	2.39	3.90	5.20	7.70	12.70
Glyceric	1.20	1.30	1.70	2.20	3.30	4.90
2-Hydroxyisobutyric	2.20	2.80	3.85	5.10	7.10	10.30
2-Hydroxybutyric	1.70	1.70	1.70	2.65	3.60	3.60
Ethylmalonic	1.00	1.00	1.20	1.80	2.60	5.20
Methylsuccinic	1.00	1.00	1.10	1.30	1.70	4.00
Adipic	1.00	1.00	1.10	1.50	2.10	5.60
Suberic	0.70	0.84	1.10	1.80	2.55	3.70
Sebasic	0.60	0.60	0.65	0.85	1.40	1.80
4-Hydroxyphenylpyruvic	1.00	1.00	1.00	1.00	2.00	7.50

Supplemental Table S2

Table S2. Bivariate correlations between measured organic acids

Organic acid	Citric	Aconitic	Isocitric	2-Keto glutaric	Succinic	Fumaric	Malic	3-Hydroxy Methyl glutaric	Lactic	Pyruvate	3-Hydroxy butyric	Pyro glutamic	2-Keto-iso caproic	2-Keto 3-methyl valeric	3-Hydroxy isovaleric	Methyl Malonic	Homo Vanillic
Citric		.586*	.579	.519	.345	-.500	.329	.423	.390	.299	.249	.236	-	.500	.266	.300	.332
Aconitic	.586		.779	.493	.368	1.000	.548	.562	.432	.299	.668	.524	-	-.500	.480	.092	.387
Isocitric	.579	.779		.507	.372	1.000	.489	.655	.445	.459	.482	.570	-	.500	.456	.224	.596
2 -ketoglutaric	.519	.493	.507		.185	1.000	.686	.351	.434	.610	.011	.257	-	.500	.274	.074	.375
Succinic	.345	.368	.372	.185		-.500	.454	.218	.102	-.035	-.076	.400	-	1.000	.222	-.115	.460
Fumaric	-.500	1.000	1.000	1.000	-.500		1.000	.500	.500	.500	-1.000	.500	-		1.000	1.000	1.000
Malic	.329	.548	.489	.686	.454	1.000		.310	.338	.483	.361	.039	-	1.000	.214	-.078	.393
3-Hydroxy 3- methylglutaric	.423	.562	.655	.351	.218	.500	.310		.273	.306	.258	.418	-	.500	.405	-.076	.429
Lactic	.390	.432	.445	.434	.102	.500	.338	.273		.651	.279	.219	-	1.000	.456	-.163	.246
Pyruvate	.299	.299	.459	.610	-.035	.500	.483	.306	.651		-.091	.112	-	1.000	.300	.011	.298
3-Hydroxy- butyric	.249	.668	.482	.011	-.076	-1.000	.361	.258	.279	-.091		.419	-	1.000	.523	-.128	.117
Pyroglutamic	.236	.524	.570	.257	.400	.500	.039	.418	.219	.112	.419		-	-.500	.496	-.027	.625
2 Ketoisocaproic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Keto 3- methylvaleric	.500	-.500	.500	.500	1.000		1.000	.500	1.000	1.000	1.000	-.500	-		-.500	-1.000	.500
3-Hydroxy- isovaleric	.266	.480	.456	.274	.222	1.000	.214	.405	.456	.300	.523	.496	-	-.500		-.020	.423
Methylmalonic	.300	.092	.224	.074	-.115	1.000	-.078	-.076	-.163	.011	-.128	-.027	-	-1.000	-.020		.211
Homovanillic	.332	.387	.596	.375	.460	1.000	.393	.429	.246	.298	.117	.625	-	.500	.423	.211	

5-Hydroxy indoleacetic	.188	.298	.504	.301	.224	1.000	.257	.355	.141	.199	.079	.315	-	-1.000	.165	.114	.460
Vanillilmandelic	.269	.400	.539	.369	.473	1.000	.334	.375	.145	.214	.182	.635	-	-.500	.371	.309	.684
4-Hydroxy- phenylacetic	.155	.428	.330	.121	.132	-.500	-.280	.139	.222	.037	.130	.407	-	-.500	.510	-.144	.192
Orotic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glutaric	0.000	.400	.800	.200	0.000		1.000	.400	-.200	-.800	1.000	0.000	-		-.200	1.000	.632
2-Hydroxy- glutaric	.427	.447	.552	.680	.454	1.000	.552	.383	.362	.420	.020	.501	-	.500	.357	.263	.677
Glycolic	.276	.236	.381	.176	.213	-1.000	-.193	.075	.462	.331	-.045	.108	-	-.500	.461	.092	.189
Oxalic	.244	.289	.430	.304	.234	1.000	.434	.274	.258	.227	-.212	.167	-		.318	.371	.362
Glyceric	.407	.449	.608	.204	.332	.500	.235	.319	.332	.170	.447	.403	-	-1.000	.510	.236	.537
2-Hydroxy- isobutyric	.262	.311	.473	.316	.242	1.000	.459	.208	.500	.492	-.129	.455	-	.500	.571	.127	.574
2-Hydroxy- butyric	1.000	-1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	-1.000	-	1.000	-1.000	-1.000	1.000
Ethylmalonic	.140	.261	.290	.268	.271	.500	.288	.168	.207	.346	.300	.358	-	-1.000	.426	.156	.363
Methylsuccinic	.246	.406	.418	.354	.222	1.000	.062	.233	.202	.134	.058	.426	-	-1.000	.380	.132	.423
Adipic	.037	.341	.311	-.258	.199	1.000	.419	-.206	.164	-.097	.831	.284	-	1.000	.441	.083	.275
Suberic	-.041	-.088	.061	-.517	.044	-1.000	-.300	.045	-.407	-.444	1.000	.122	-	1.000	-.070	-.545	-.124
Sebasic	.800	.200	-.600	-.400	.800		1.000	-.800	.800	.800	1.000	.400	-		1.000	-1.000	.400
4-Hydroxy- phenylpyruvic	-.358	.379	.111	.011	.018		.344	.449	.475	-.084	.564	.819	-		.432	-.311	-.196

*In bold are highlighted the statistically significant associations between metabolites ($p < 0.05$).

-. Negligible values < 0.00

Table S2. Bivariate correlations between measured organic acids.

Organic acid	5-Hydroxy indole acetic	Vanillin mandelic	4-Hydroxy phenyl acetic	Orotic	Glutaric	2-Hydroxy glutaric	Glycolic	Oxalic	Glyceric	2-Hydroxy Iso-butyric	2-Hydroxy butyric	Ethyl malonic	Methyl succinic	Adipic	Suberic	Sebacic	4-Hydroxy Phenylpyruvate
Citric	.188	.269	.155	-	0.000	.427	.276*	.244	.407	.262	1.000	.140	.246	.037	-.041	.800	.423
Aconitic	.298	.400	.428	-	.400	.447	.236	.289	.449	.311	-1.000	.261	.406	.341	-.088	.200	.562
Isocitric	.504	.539	.330	-	.800	.552	.381	.430	.608	.473	1.000	.290	.418	.311	.061	-.600	.655
2 -Ketoglutaric	.301	.369	.121	-	.200	.680	.176	.304	.204	.316	1.000	.268	.354	-.258	-.517	-.400	.351
Succinic	.224	.473	.132	-	0.000	.454	.213	.234	.332	.242	1.000	.271	.222	.199	.044	.800	.218
Fumaric	1.000	1.000	-.500	-		1.000	-1.000	1.000	.500	1.000		.500	1.000	1.000	-1.000		.500
Malic	.257	.334	-.280	-	1.000	.552	-.193	.434	.235	.459	1.000	.288	.062	.419	-.300	1.000	.310
3-Hydroxy 3- methylglutaric	.355	.375	.139	-	.400	.383	.075	.274	.319	.208	1.000	.168	.233	-.206	.045	-.800	1.000
Lactic	.141	.145	.222	-	-.200	.362	.462	.258	.332	.500	1.000	.207	.202	.164	-.407	.800	.273
Pyruvate	.199	.214	.037	-	-.800	.420	.331	.227	.170	.492	1.000	.346	.134	-.097	-.444	.800	.306
3-Hydroxybutyric	.079	.182	.130	-	1.000	.020	-.045	-.212	.447	-.129	1.000	.300	.058	.831	1.000	1.000	.258
Pyroglutamic	.315	.635	.407	-	0.000	.501	.108	.167	.403	.455	-1.000	.358	.426	.284	.122	.400	.418
2-Ketoisocaproic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Keto 3methylvaleric	-1.000	-.500	-.500	-		.500	-.500		-1.000	.500	1.000	-1.000	-1.000	1.000	1.000		.500
3-Hydroxyisovaleric	.165	.371	.510	-	-.200	.357	.461	.318	.510	.571	-1.000	.426	.380	.441	-.070	1.000	.405
Methylmalonic	.114	.309	-.144	-	1.000	.263	.092	.371	.236	.127	-1.000	.156	.132	.083	-.545	-1.000	-.076
Homovanillic	.460	.684	.192	-	.632	.677	.189	.362	.537	.574	1.000	.363	.423	.275	-.124	.400	.429
5- Hydroxyindoleacetic		.433	.084	-	-.200	.303	-.018	.228	.372	.279	-	.211	.356	.210	.002	.200	.355

Vanillilmandelic	.433		.113	-	.800	.587	.095	.394	.450	.488	-1.000	.460	.531	.232	.086	0.000	.375
4-Hydroxyphenylacetic	.084	.113		-	-.800	.189	.243	.135	.308	.231	-1.000	.088	.371	.207	-.320	-.400	.139
Orotic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glutaric	-.200	.800	-.800	-		.400	0.000	.200	.400	-.200		-.105	-.400	-.500	1.000	-	.400
2-Hydroxyglutaric	.303	.587	.189	-	.400		.209	.499	.242	.558	1.000	.448	.452	-.170	-.406	-.200	.383
Glycolic	-.018	.095	.243	-	0.000	.209		.094	.318	.388	-1.000	.051	-.007	-.050	-.161	.800	.075
Oxalic	.228	.394	.135	-	.200	.499	.094		.379	.423		.293	.339	-.001	-.237	1.000	.274
Glyceric	.372	.450	.308	-	.400	.242	.318	.379		.383	-1.000	.421	.377	.360	-.068	.400	.319
2-Hydroxyisobutyric	.279	.488	.231	-	-.200	.558	.388	.423	.383		1.000	.571	.509	.277	-.233	.800	.208
2-Hydroxybutyric		-1.000	-1.000	-		1.000	-1.000		-1.000	1.000		-1.000	-1.000	1.000	1.000	-	1.000
Ethylmalonic	.211	.460	.088	-	-.105	.448	.051	.293	.421	.571	-1.000		.653	.420	-.409	1.000	.168
Methylsuccinic	.356	.531	.371	-	-.400	.452	-.007	.339	.377	.509	-1.000	.653		.445	-.198	-.400	.233
Adipic	.210	.232	.207	-	-.500	-.170	-.050	-.001	.360	.277	1.000	.420	.445		.370	.500	-.206
Suberic	.002	.086	-.320	-	1.000	-.406	-.161	-.237	-.068	-.233	1.000	-.409	-.198	.370		-.400	.045
Sebasic	.200	0.000	-.400	-		-.200	.800	1.000	.400	.800		1.000	-.400	.500	-.400		-.800
4-Hydroxyphenyl pyruvic	.190	-.090	.359	-	.500	-.158	-.190	-.058	.363	.111		-.288	-.224	.866	1.000		.449

*In bold are highlighted the statistically significant associations between metabolites ($p < 0.05$).

-: Negligible values < 0.00

APPENDIX 6B UNPUBLISHED WORKS

Statistics plays an important role in nutrition research. The 4-year PhD study entailed an intensive course on statistics including software and its application in the field of nutrition. The following manuscript is the result of the many tedious hours devoted to the learning of statistics.

Title: A comprehensive study of assessing a distribution's normality. Availability of statistical methods in nine software tools.

Maria Michelle Papamichael¹; Eleni Maria Theodoraki²

¹School of Allied Health, Human Services & Sport, Department of Dietetics, Nutrition and Sport, La Trobe University, Melbourne, Victoria, Australia

²1st Propedeutic Surgery Clinic, Department of Biostatistics, National & Kapodistrian University of Athens, Greece

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Corresponding Author:

Maria Michelle Papamichael, School of Allied Health Human Services & Sport, Department of Dietetics, Nutrition and Sport, La Trobe University, Melbourne Vic., 3086, Australia. E-mail: sassipap@hotmail.com

ABSTRACT

Normality is one of the assumptions required in parametric tests and violations may lead to invalid inferences. Theory-driven or descriptive graphical and numerical methods can be applied to assess if a distribution is approximately normal. The purpose of this article is to present tools of normality assessment available in nine statistical software. Comparisons are made among methods regarding sample size and shape of distributions as well as among software concerning availability and user-friendliness. Finally, an overall method of decision-making on normality is proposed.

Keywords Normal distribution; normality testing; software; statistical tests

1.1 INTRODUCTION

Normality is a term which is widely used in statistics referring to a distribution and therefore may be applied to quantitative variables (Chan 2003). The normal distribution can be described using measures of central tendency, measures of spread and measures of symmetry (Shahravan 2013). Why is the normal distribution so important? Parametric tests (t-test, F-test, Analysis of Variance (ANOVA), correlation, regression etc.) rely on specific assumptions one of them being normality (Altman and Bland 1995). Prior to data analysis, it is important that this assumption is investigated in order to ensure that the correct statistical test is applied (Shahravan 2013). If the normality assumption is violated then interpretation and inferences may not be reliable and valid.

Parametric tests are preferred to analyze quantitative variables because they are robust, have more ‘power’ and require less data to make a stronger conclusion than non-parametric tests (Neideen 2007). According to Hoekstra (2012), very few authors of published articles reported whether normality assumptions were checked and satisfied (Hoekstra 2012). Many researchers conveniently assume normality based on the Central Limit Theorem which states that, for non-normal data the distribution of the sample means has an approximate normal distribution regardless the shape of the distribution, provided that the sample is large (at least 30) and all samples have the same size. So, for small sample size what should we do? Furthermore, in the case of sample size (less than 30), formal tests of normality have low power in detecting deviations from normality (Razali 2011) and the estimate of the means will approach the normal distribution only when the population distribution is normal (Pagano 2006).

The purpose of this article is to present all statistical tools of normality assessment applied with nine statistical software in various sample sizes, provide comparisons using transformations in real dietary data and propose an overall method of decision-making. Therefore graphical and numerical methods for normality assessment are analyzed and results of the statistical software’s usefulness are evaluated. Mathematical explanation and compilations are beyond the scope of this article.

1.2 METHODS

Definitions of terms used in statistics are presented below in order to reinforce understanding of statistical terms referred to in this article:

Measures of central tendency The mean, median and mode are measures of central tendency. The mean is the average number of data values. The median (50th percentile) is the middle of ranked values where 50% of observations lie above this point and 50% below. If the number of data values is odd then the median is the middle ranked value, whereas in the case of even, the median is the average of the two center ranked values. The 25th percentile and the 75th percentile describe the value below which percentage of observations lie respectively. The mode is the data value which occurs most frequently. If the mean and median are equal, then the data is usually symmetrical (Chan 2003); (Overholser and Sowinski 2007).

Measures of dispersion The standard deviation and the variance are referred to as measures of dispersion. The variance which is the square of the standard deviation reveals how observations of a random variable deviate from the mean (Driscoll, Lecky, and Crosby 2000). The standard deviation determines the height and width of the curve (Shahravan 2013).

Measures of symmetry Kurtosis and skewness provide information about the shape and symmetry of the distribution (Chan 2003). Skewness measures deviation from the symmetrical normal curve (DeCarlo 1997). Kurtosis refers not only to the “peakedness” or “pointiness” of the bell-shaped curve, but also to the tails (Chan 2003; Shahravan 2013). Left skewness is indicated by a negative coefficient value ($\text{skew} < 0$) and right skewness by a positive coefficient value ($\text{skew} > 0$). Positive kurtosis indicates long, heavy tails with a higher peak than the normal distribution, whereas negative kurtosis describes a distribution with short, light tails and a flat peak (DeCarlo 1997; Chan 2003).

Outliers are extreme (unusually large or small) values which vary from the mean value of the sample distribution (DeCarlo 1997).

The Null Hypothesis The null hypothesis (H_0) for all statistical tests which assess normality is, that the data distribution is normal (Razali 2011). The p -value is the probability of a test

statistic occurring if the null hypothesis is true. If the significance level is considered to be α , then P values that are larger than α , mean that, there is a large probability that the null hypothesis is not rejected. However, P values that are smaller than α , lead to rejection of the null hypothesis since there is a small probability that the null hypothesis is true (Peat and Barton 2005). In statistics a value of 0.05 or 0.01 is often used for α .

Power of a statistical test A test's power is the probability of correctly rejecting the null hypothesis when it is false. In the case of normality, the term "power" measures the ability of a test to detect whether a sample comes from a non-normal distribution (Razali 2011).

1.2.1 Graphical methods

Graphical methods give a visual presentation of the data distribution for a random variable and compare this distribution to a theoretical one. In this section normality will be explained by visual presentation using descriptive and theory-driven plots.

1.2.1.1 Descriptive plots

Histogram Histograms can be used to represent quantitative data from a large sample population (Krzywinski 2014). The histogram is a frequency distribution which plots the observed values against their frequency. The histogram presentation provides information about the mean, standard deviation, mode, symmetry, shape of the data distribution and whether the distribution is bell-shaped. Normality is assumed when the histogram presentation approximates a symmetrical, bell-shaped curve having most data values in the center and values tailing off evenly in either direction (Peat 2005). If the shape is skewed then non-normality can be confirmed (Shahravan 2013). Deviations at the end of the curve indicate the presence of outliers.

Stem-leaf Plot In a stem and leaf plot the exact values of the distribution are displayed. The whole numbers are displayed as the stem and the ones as the leaf. The stem and leaf are separated by a vertical line. Firstly, the numbers in the group must be arranged in order from the smallest to the largest. The stem is formed by displaying the ten digits left of the vertical line and the leaves by displaying each ones digit right of the vertical line (Pérez-Vicente 2009). In the case of decimal numbers, the whole number will be displayed on the left of the vertical line, the decimal point will be represented by the vertical line and the number after the decimal point will be placed on the right of the vertical line (Tyler 2013).

Dot Plot The dot plot is useful in displaying small sample sizes (Krzywinski 2014). In the dot plot, individual data values are presented simply as points. For each value in the data set, a dot is placed above that value on the x-axis. If a value occurs more than once, the dot is stacked above that value. Normality is indicated when the arrangement of dots form the characteristic bell-shaped curve (Driscoll, Lecky, and Crosby 2000).

Box-Plot Box-plots give information regarding the shape, variability and center or median of a data set. Box plots can be created from small sample sizes and provide information about the tails of a distribution. They are particularly useful for displaying skewed data. A box plot provides a pictorial presentation as the bottom of the box represents the 25th percentile, the top of the box the 75th percentile. The 50th percentile or median is the line which lies exactly in the middle of the box. The interquartile range is the range between the 25th and the 75th percentile. More specifically, it is the length of the box. The interquartile range shows the location of most of the data. Whiskers are the lines extending from the ends of the box (Peat and Barton 2005) and represent the minimum and maximum values in the data set. But in the case when outliers are present, the whisker will represent the maximum and minimum values when they are within 1.5 times above or below the interquartile range (Peat and Barton 2005); (Cleveland and McGill 1985);(Driscoll, Lecky, and Crosby 2000). Data points that are more than 1.5 times the interquartile range are called outliers and are indicated in the plot as open circles (Peat and Barton 2005). Extreme outliers are more than 3 times the interquartile range and are usually shown as asterisks (Peat and Barton 2005). The presence of many outliers can cause skewness indicating non-normality (Peat and Barton 2005);(Cleveland and McGill 1985). A boxplot that is symmetric will have the median line at approximately the centre of the box and symmetric whiskers on either side of the box which will suggest that the data may come from a normal distribution (Peat and Barton 2005); (Cleveland and McGill 1985). One disadvantage of the box-plot is that it does not convey information about the actual sample size (Krzywinski 2014).

1.2.1.2 Theory-driven plots

Probability-Probability (P-P)/ Quantile-Quantile (Q-Q) Plots For data that are not normal, the Q-Q and P-P plots can help determine how data deviate from normality. These two plots indicate if the data are tailed, skewed, or if kurtosis exists (Lund 2013). The main differences between these two plots are that the P-P plot identifies deviations in the middle of the

distribution, whereas the Q-Q plot shows deviations in the tails (SAS 1999). The Q-Q plot is a probability plot which compares the quantiles of a data distribution with the quantiles of the standard normal distribution (Park 2008). The P-P plot compares an empirical cumulative distribution function of a variable with the standard normal distribution function. For both types of plots if the data distribution matches the theoretical normal distribution, then the points on the plot will lie on the diagonal line and form a straight line, skewness and kurtosis will have a value near zero and it can be concluded that the distribution seems to be approximately normal. If normality is violated the points will form a curve instead of a straight line. If the plotted points appear to curve upwards or to be concave up (U-shaped), then the data is said to be skewed to the left ($\text{skew} < 0$). If the plotted points curve downwards (upside down U or bow-shaped) or concave down, then the data is said to be right skewed ($\text{skew} > 0$). If the left end of the point pattern lie below the normal line and the right end is above the line then this indicates the presence of long tails. But if the left end is above the normal line and the right end is below the line this can be interpreted as short tails. Extreme values at the end of the curve indicate the presence of outliers (Chan 2003; Lund 2013).

Kernel density estimation A kernel distribution is a non-parametric representation of the probability density function (pdf) of a random variable. This distribution is an improvement of the histogram. It is defined by a smoothing function and a bandwidth value which determines the smoothness of the density curve. Similar to a histogram, the kernel distribution builds a function to represent the probability distribution using the sample data. However, unlike the histogram which places the data value in discrete bins, a kernel distribution sums the component smoothing functions for each data value to produce a smooth, continuous probability curve (Mathworks 2016; Salgado-Ugarte, Shimizu, and Taniuchi 1994). In the case of normality, the kernel density plot will display a symmetrical unimodal bell-shaped curve (Silverman 1986). Kernel density estimation is a useful tool for the exploratory stage in data analysis. Density estimates are valuable because they can reveal skewness, heavy or light tails and multimodality in data (Silverman 1986).

1.2.1.3 Comparison of graphical methods

From graphical methods, the histogram is the first and easiest way to assess if the data distribution is normal and is applicable for all sample sizes. The shape of the histogram provides information about skewness, kurtosis, and the presence of outliers, from which normality can be assessed immediately (Chan 2003) For small sample sizes, stem-leaf plots and dot plots are

useful for summarizing quantitative variables whereas for large samples box-plots, Q-Q plots and P-P plots are useful (Park 2008). The P-P plots and box-plots are excellent graphs for revealing the presence of outliers (Pfahler 2014).

1.2.2 Numerical methods

Graphical methods rely on the expertise and subjectiveness of the researcher and therefore interpretations are a matter of judgment. Numerical methods include descriptive statistics and statistical tests which complement the visual presentation.

1.2.2.1 Descriptive statistics

Measures of central tendency Normality is assumed if the value for mean, median and mode are approximately equal (Chan 2003) (Peat and Barton 2005).

Measures of symmetry A normally distributed variable will have skewness and kurtosis near zero. The acceptable range for normality is kurtosis and skewness lying between -1 to 1 which indicates that the distribution has an approximately normal bell- shaped curve (Chan 2003) (Shahravan 2013). Values around -2 to +2 indicate a reasonable degree of skewness and kurtosis, whereas values below -3 and above +3 show that there is a significant degree of skewness and peakedness or flatness of the curve and therefore inferring that the data are not normally distributed (Peat and Barton 2005). It should be noted that these measures are affected by sample size (Razali 2011).

1.2.2.2 Theory driven statistics

Unlike parametric tests which are based on assumptions, non-parametric tests do not rely on any distribution and therefore are applicable for a wide range of continuous distributions such as the Weinbull, exponential, gamma, beta, logistic, uniform, pareto, student's t, including normal and log- normal distributions. These non-parametric goodness-of-fit tests are used to assess whether data are consistent with a hypothesized null distribution (Arnold and Emerson 2011).

Parametric statistical tests can be applied in order to establish whether or not the given sample comes from a normally distributed population. The result of the statistical test (p -value) will indicate whether the null hypothesis (H_0) should be rejected or not (Razali 2011). To our knowledge, formal statistical tests that are used to assess normality are:

Kolmogorov-Smirnov Test The Kolmogorov-Smirnov normality test was originally devised for use when the mean and standard deviation of the sample are known (Kolmogorov 1933). Most software packages use the Kolmogorov-Smirnov test with Lilliefors correction for assessment of normality in the case of mean and standard deviation unknown (Lilliefors 1967). Ghasemi recommend that Kolmogorov-Smirnov be used for sample sizes larger than 50 (Ghasemi and Zahediasl 2012).

Anderson-Darling Test The Anderson-Darling test is a modification of the Kolmogorov-Smirnov test which is more sensitive to discrepancies at the tails of the distribution than the Kolmogorov-Smirnov test (Anderson 1954)(Stephens 1974). This statistical test should be used when distributions are tailed and for sample size larger than 2000 (Park 2008).

Ryan-Joiner Test The Ryan-Joiner test is based on the correlation between the data sample and the data expected from a normal distribution. The Ryan-Joiner statistic assesses the strength of this correlation. The correlation is a measure of the strength of a linear relationship, with the sign of the correlation indicating the direction of the relationship. A positive correlation coefficient indicates increasing relationship and a negative correlation for decreasing. The correlation coefficient may vary from -1 to +1 and if the correlation coefficient approaches 1, then the population is likely to be normal. The Ryan-Joiner test is similar to the Shapiro-Francia and Shapiro-Wilks tests. According to Ryan and Joiner (1976), the Ryan-Joiner test is superior to Shapiro-Francia for long and heavy tailed distributions (Ryan 1976).

Cramer Von Mises Test The Cramer-Von Mises test is similar to the Kolmogorov-Smirnov test and is applicable for large sample size (PETTITT 1976; Park 2008; Steele 2005).

Shapiro-Wilk Test The Shapiro-Wilk test calculates a W statistic that tests whether a random sample originates from a normal distribution (Shapiro 1972). Small values of W are evidence of departure from normality (Shapiro 1965). The original Shapiro-Wilks (1965) is valid for sample sizes between 3 and 50 and is recommended for non-aggregated, ungrouped and not

skewed data (Gould 1991). A new approximation of the Shapiro-Wilk test (the Shapiro-Wilk W test) was devised by Royston (1982) and the test is applicable for sample sizes ranging from 4 to 2000 (Royston 1982; Royston 1992). Lund and Ghasemi recommend that Shapiro-Wilk be applied for sample sizes less than 50 (Ghasemi and Zahediasl 2012; Lund 2013).

Shapiro-Francia test The Shapiro-Francia W' test is based on the original Shapiro-Wilk test and is applicable for very large samples from 5 to 5000 and also for data which are non-aggregated or not skewed (Shapiro 1972) (Royston 1983) (Gould 1991).

Skewness – Kurtosis tests Skewness and kurtosis tests are normality tests which are based on two tests: a test for skewness and a test for kurtosis (D'Agostino 1990) (Royston 1991). The null hypothesis for both tests being that the distribution is normal when skewness and kurtosis are equal to zero (D'Agostino 1990).

D'Agostino–Pearson test The D'Agostino-Pearson test, also known as an omnibus test, is a powerful test which is able to identify non-normality based on skewness and kurtosis. This test combines the skewness and kurtosis statistics and the D'Agostino-Pearson K^2 statistic to produce a powerful and informative test which is applicable for all sample sizes (D'Agostino 1973; D'Agostino 1990). Also the Royston improved D'Agostino test is based on the D'Agostino test with the correction proposed by Royston, (1991) and is applicable for sample size larger than 8 (Royston 1991).

Jarque-Bera test The Jarque-Bera test is a normality test which is able to identify non-normality due to skewness and kurtosis. This test is also based on the skewness and kurtosis sample coefficients (Jarque 1987). The Jarque–Bera test is applicable for large sample size ($N > 2000$) with symmetric distributions having medium or long tails and for unsymmetrical slightly skewed distributions with long tails (Thadewald 2007) (Jarque 1987) (Park 2008).

Chi-Square Test The Chi-Square Test, a non-parametric test can be used to test the hypothesis that the data is normally distributed and is used for grouped data (Snedecor 1989). Application of this test requires that each group contains at least 5 values and that the sample size is equal to at least five times the number of groups (Romeu 2003).

Skewness-Kurtosis tests	-	√	√ ^a	-	-	-	-	-	-
Shapiro-Wilks	√	√	√	-	√	√	√	√	√
Shapiro-Francia	-	√	-	-	√	-	-	√	√
K-S Lilliefors	√	-	√	√	√	√	√	√	√
Cramer-von Mises	√	-	-	-	-	-	-	√	√
Anderson-Darling	√	-	-	√	-	-	√	√	√
Ryan-Joiner	-	-	-	√	-	-	-	-	√
Chi-Square normality test	-	-	-	-	√	-	-	√	√

√: statistical test available in the particular software

- : statistical test not available

^aA SPSS Macro for testing skewness and kurtosis (DeCarlo 1997).

^bA SAS macro is available in the SAS library on the Web (D'Agostino 1990).

^cA SPSS syntax is available for kernel density estimation plots (Howell 2010).

^dA macro is available for kernel density estimation plots in Minitab from the Royal Society of Chemistry, U.K (RSC 2016a).

^eAn add-in is available for Excel from the Royal Society of Chemistry, UK (RSC 2016b).

As far as the statistical software packages are concerned, all graphical methods are available in Stata, SAS and R. R package provides all available statistical tests (9 in number), Matlab, MedCalc, SAS five, followed by XLSTAT applying four, STATA and Minitab three and finally SPSS and Statistica only two.

1.3 APPLICATION

With the aim to demonstrate a decision making path in the case of small sample size (less than 30), the normality assumption was investigated using a quantitative variable (Body Mass Index (BMI)) from a study assessing the diet quality of Greek adolescents. From a total of 246 male and female students aged 12-17.5 y.o participating from high schools in Greece, a small (Razali 2011) subsample of 20 students was used. In graphical methods descriptive and theory-driven plots are presented, followed by the measures of central tendency, dispersion, skewness and kurtosis in descriptive statistics, and finally statistical analysis of BMI. All descriptive statistics and graphical methods, except kernel density plots were executed with SPSS (Version 20) IBM software. Kernel density plots were estimated according to Wessa (2015)(Wessa 2015). Regarding all statistical tests, XLSTAT 14 provided a broader range of tests in comparison to SPSS and for this reason XLSTAT 14 was used (Addinsoft 2015).

In the application below, graphical and descriptive methods revealed that the variable BMI was right-skewed and non-normal (Figs. 1a-1g).

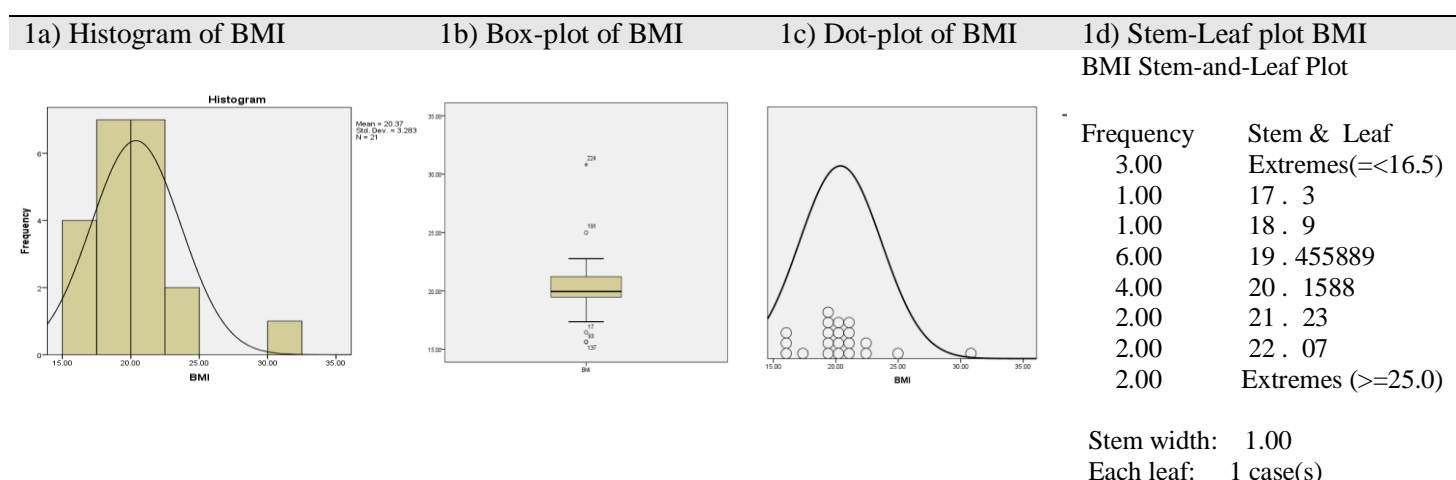


Fig. 1 a) Histogram of variable BMI (N=20)

Fig. 1 b) Box-plot of variable BMI

Fig. 1 c) Dot-plot of variable BMI

Fig. 1 d) Stem-Leaf plot of variable BMI

From the box-plot in Fig. 1b) it is evident that the median is not centrally located in the box and that the whiskers are not symmetric on either side of the box which suggests that the data are not normally distributed. Data points that are 1.5 times the interquartile range (that is observations number 17, 93, 137 and 191) are the outliers, whereas observation number 224 (shown by the asterisk) is the extreme outlier. The presence of outliers causes the distribution to be skewed (Peat and Barton 2005).

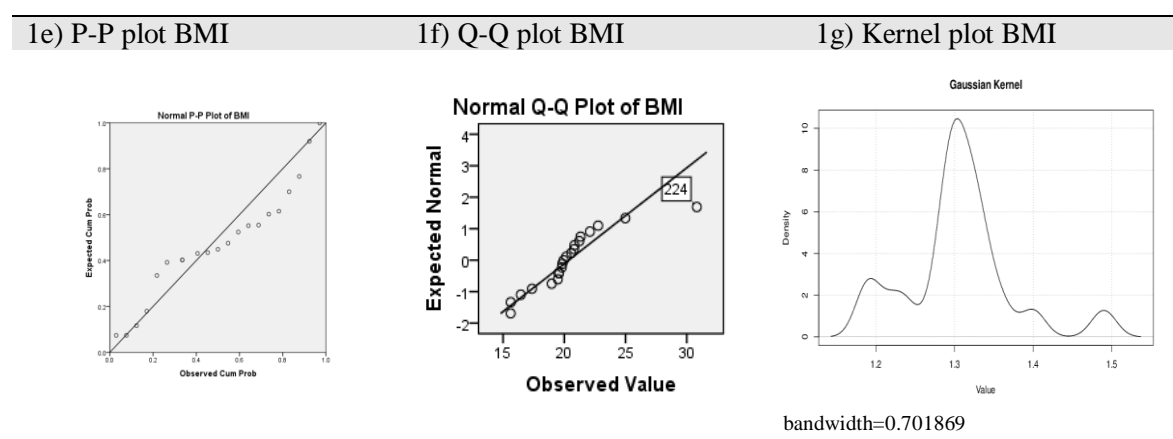


Fig. 1e) P-P plot variable BMI

Fig. 1f) Q-Q plot variable BMI

Fig. 1g) Kernel plot variable BMI

In Fig. 1e) the P-P plot shows that data points do not lie on the normal line but extend below the normal line indicating positive skewness.

In Fig. 1f) The Q-Q plot for BMI deviates from the normal line. The data distribution forms a slightly curved line which extends below the normal line (concave downwards) indicating positive skewness which is right-tailed. The data point (observation number 224) which lies outside from the majority of points is the outlier.

In Fig. 1g) The kernel density plot for BMI illustrates a multi-modal distribution as represented by the multiple peaks, indicating non-normality.

Descriptive statistics for the variable BMI reveal that, the mean, median and mode values are near, whereas the measures of skewness and kurtosis lie outside the range of -1 to 1 indicating asymmetry (Table 2a). More specifically, the measure of skewness is greater than zero, showing a right skewed distribution and a value of kurtosis close to three indicates that the curve is highly-peaked. Therefore, from descriptive statistics BMI seems to be not normal.

Table 2a: Descriptive Statistics for BMI

	BMI
Mean	21.91
Median	22.12
Mode	22.86
Std. deviation	3.67
Skewness	1.29
Kurtosis	2.66

*Value of kurtosis is given as excess kurtosis which is kurtosis-3

As mentioned earlier, for an overall view of assessing normality statistical tests should be applied (Table 3a).

Table 3a. Statistical Analysis of BMI: Tests of Normality

	N	Kolmogorov-Smirnov	Chi-Square (GOF)	Shapiro-Wilks	Anderson-Darling	Kolmogorov-Smirnov ¹	Jarque-Bera
BMI	20	0.41	0.00	0.01	0.02	0.05	0.00

*GOF-Goodness of Fit test

¹ Kolmogorov-Smirnov test with Lilliefors correction

In Table 3a), for small sample size ($n=20$), based on all tests except from the Kolmogorov-Smirnov tests, the normality assumption is rejected at the 5% significance level ($p<0.05$) and hence it may be assumed that BMI is not normally distributed.

However, both Kolmogorov-Smirnov tests show $p > 0.05$ indicating not to reject the null hypothesis. A plausible explanation is that for small sample size the Kolmogorov-Smirnov test has low power, performing Type II error (Razali 2011). Hence, based on both graphical and numerical methods it may be concluded that variable BMI seems to be non-normal.

Due to the fact that data are positively skewed, and to reduce bias caused by outliers a logarithmic transformation was performed (Bland 1996). Then graphical methods and descriptive statistics were applied for the new variable Log (BMI) using SPSS (Version 20) (IBM) and XLSTAT 14 was used to execute statistical tests (Figs.2a-2g).

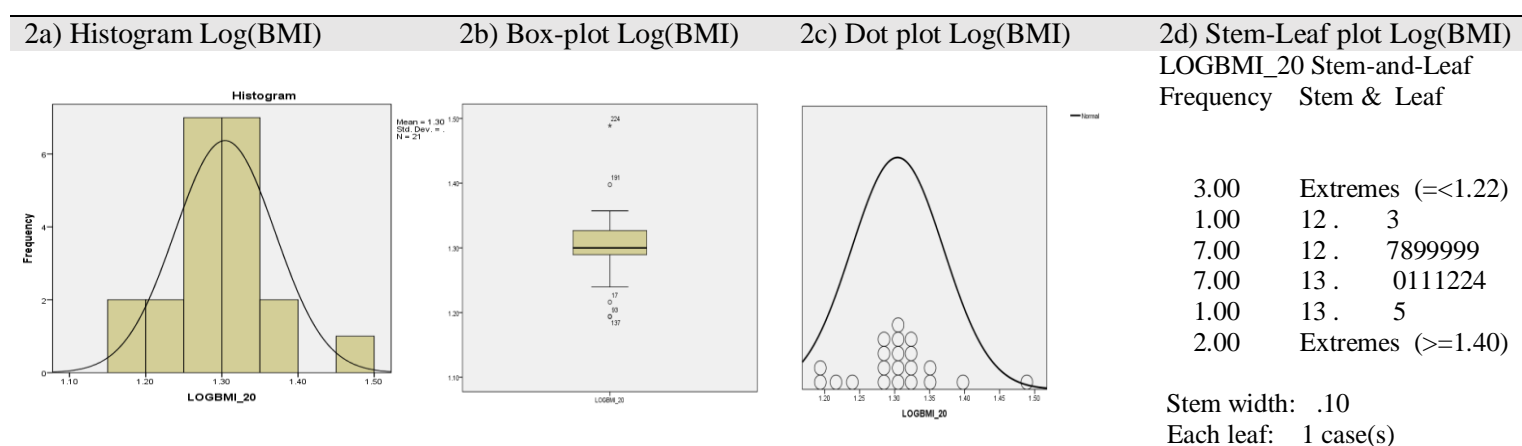


Fig. 2a) Histogram of variable Log (BMI) ($N=20$)

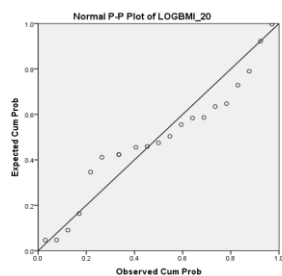
Fig. 2b) Box-plot of variable Log (BMI)

Fig. 2c) Box-plot of variable Log (BMI)

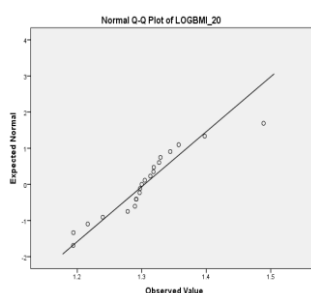
Fig. 2d) Stem-Leaf plot of variable Log (BMI)

The graphical presentation of Log (BMI) in Figs. 2a-2d show an approximate symmetrical bell-shaped near normal curve. Although outliers are visualized in the box-plot, it does not seem to affect the symmetry of the distribution. The whiskers seem to be fairly symmetrical around the box and the median close to the middle of the box.

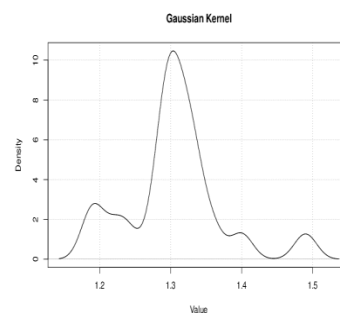
2e) P-P plot Log(BMI)



2f) Q-Q plot Log(BMI)



2g) Kernel plot Log(BMI)



Bandwidth= 0.015679

Fig. 2e) P-P plot variable Log (BMI)**Fig. 2f)** Q-Q plot variable Log (BMI)**Fig. 2g)** Kernel plot variable Log (BMI)

However, the plots outlined in Figs. 2e), 2f) and 2g) show a non-normal distribution. From the P-P and Q-Q plot it is evident that the data points do not exhibit linearity, but illustrate a slightly skewed distribution. In addition, the kernel density plot shows a multi-modal distribution.

Contrastingly, descriptive statistics show that after logarithmic transformation the values for mean, median and mode of Log (BMI) are approximately equal and that the measures of skewness and kurtosis lie within the range of -1 to 1, therefore it may be deduced that Log(BMI) seems to be approximately normal (Table 2b).

Table 2b): Descriptive Statistics for Log (BMI)

	LOG(BMI)
Mean	1.33
Median	1.34
Mode	1.36
Std. deviation	0.07
Skewness	0.77
Kurtosis	1.10

*Value of kurtosis is given as excess kurtosis which is kurtosis-3

Statistical analysis of Log (BMI) showed that all statistical tests computed p-values greater than 0.05, accepting the null hypothesis (Table 3b). Hence it may be assumed that Log (BMI) is normally distributed.

Table 3b): Statistical analysis of Log (BMI): Tests of Normality

	N	Kolmogorov-Smirnov	Chi-Square (GOF)*	Shapiro-Wilks	Anderson-Darling	Kolmogorov-Smirnov ¹	Jarque-Bera
Log BMI	20	0.62	0.13	0.09	0.06	0.18	0.27

*GOF-Goodness of Fit test

¹ Kolmogorov-Smirnov test with Lilliefors correction

Summarizing the results, normality assessment of real data using both graphical and numerical methods showed that the quantitative variable BMI was not normally distributed. However, after logarithmic transformation, the variable Log (BMI), approached normality as was suggested from the outcome of statistical tests, descriptive statistics and most of the graphical presentations.

1.4 CONCLUSION

Normality is an assumption which must be satisfied before the application of parametric tests. For this purpose the authors suggest that both graphical and numerical methods are applied especially in the case of small sample sizes where statistical tests have low power and may be misleading. Firstly, histograms should be utilized in order to provide information about the shape and symmetry of the data distribution, followed by P-P plots, Q-Q plots and box-plots which are useful in identifying deviations from normality due to the presence of outliers. At the same time, descriptive statistics should be taken into consideration to reinforce the visual assessment. Last but not least, the shape of the data distribution and the sample size will determine which statistical test should be applied to test the null hypothesis. If the distribution is symmetrical or slightly unsymmetrical and for all sample sizes, it is recommended that the Shapiro-Wilk or Shapiro-Francia is applied. For distributions showing non-normality due to skewness and kurtosis and for all sample sizes, the D'Agostino- Pearson and D'Agostino-Pearson K^2 tests are the tests of choice. Finally, if D'Agostino Pearson is not available then in the case of symmetric long-tailed distributions, Jarque-Bera test should be adopted and in the case of asymmetric tailed distributions, Anderson-Darling test is a good alternative.

Appropriate transformations towards normalization are suggested when non normality is concluded.

Comparing software, R, Matlab, Stata and SAS were mathematically orientated or knowledge of computer programming was needed for execution of syntax and therefore more difficult in practice. Although most software provided all plots, Minitab was the quickest (with one “click”) and MedCalc was found to be more straight-forward and easier in execution of statistical tests. The authors recommend that Minitab is chosen for execution of graphical methods whereas for statistical tests, MedCalc is preferred. Hopefully, a better understanding of the concept of normality will result in the use of appropriate statistical tests, in the production of valid results and interpretations.

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APPENDIX 6B UNPUBLISHED WORKS

Thanasoula M, Sarandi E, Anamaterou C, Papakonstantinou E, Geraci F, **Papamichael M.M.**, Itsiopoulos C, Tsoukalas D, **Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases** in Makowski G. (ed) *Advances in Clinical Chemistry* (Elsevier).

Metabolic profiling of organic and fatty acids in chronic and autoimmune diseases

Maria Thanasoula^{1*}, Evangelia Sarandi^{1,2*}, Chrisanthi Anamaterou¹, Evangelos Papakonstantinou¹, Franco Geraci³, Maria Michelle Papamichael⁴, Catherine Itsiopoulos⁴, Dimitris Tsoukalas^{1,3}

¹ Metabolomic Medicine Clinic, 10674 Athens, Greece

² Laboratory of Toxicology and Forensic Sciences, Medical School, University of Crete, 71003 Heraklion, Greece

³ European Institute of Nutritional Medicine, E.I.Nu.M, 00198 Rome, Italy

⁴ La Trobe University, School of Allied Health, Department of Rehabilitation, Nutrition & Sport, Melbourne, Australia

*contributed equally

Abstract

Metabolomics is a powerful tool for the identification and validation of biomarkers in several autoimmune and chronic diseases. Several parameters affect the metabolite profile from the population characteristics to the selection of analytical method. In the current chapter we summarize the main analytical methods and results of the metabolic profiling of fatty and organic acids performed in human metabolomic studies for asthma, COPD, psoriasis and Hashimoto's thyroiditis. We discuss the most significant metabolic alterations associated with these diseases, after comparison of either a single patient's group with healthy controls, or several patient's subgroups of different disease severity and phenotype with healthy controls, or of a patient's group before and after treatment. Finally, we present key metabolic patterns that are associated with each disease and their potency for the unraveling of disease pathogenesis, prediction, diagnosis, patient stratification and treatment selection.

Introduction

Metabolomics in the identification and validation of clinical biomarkers

Metabolomics is the study of small molecules, called metabolites, that are generated from cellular metabolic activity, serve as the substrates, intermediates, and products of cellular pathways and are the final products of gene expression under the influence of microbiome, environment and lifestyle. As such, metabolites are a direct, functional reflection of the biochemical processes occurring in a particular phenotype and their pattern can be the diagnostic representation of the disease. Therefore, metabolomics is the closest field to phenotype expression and it reflects the various interactions between the genome and the environment, occurring also in many Non-Communicable Diseases (NCDs) including chronic and autoimmune diseases, such as asthma, Chronic Obstructive Pulmonary Disease (COPD), psoriasis and Hashimoto, all discussed in this book. NCDs are by far the leading cause of death worldwide and in 2016, they were responsible for 71% (41 million) of the 57 million deaths which occurred globally and is expected to increase rapidly the next years [1,2]. This is mainly due to the extended lifespan the last decades and the modern lifestyle that includes lack of physical activity, increased stress factors, as well as diet critically low in nutritional value, all factors affecting the metabolism and favoring the development of chronic and autoimmune diseases [2]. Since the number of incidents is gradually increasing the need for prevention that will significantly improve the lifestyle and the need for personalized treatment that will ameliorate the symptoms and increase the quality of everyday life of patients with chronic or aging-related diseases is crucial. The role of metabolomics in medical research has continued to evolve and the metabolome has been increasingly recognized as an essential aspect of our understanding of human disease. It is used in various diseases to study a large number of sample types including urine, plasma and serum, skin samples, cerebrospinal fluid, exhaled breath condensate (EBC), bronchoalveolar lavage fluid (BALF) and saliva. Urine and blood are the most commonly used biofluids for metabolomic studies as both can be easily collected and contain a large number of detectable metabolic features [3]. The relationship between human health and the metabolome is driven by the ability of small shifts in biochemical pathways to produce dramatic changes in cellular metabolites. In particular, fatty acids (FAs) and organic acid (OAs) metabolomic profiling has gained great scientific interest the recent years [4]. FAs play essential roles in several biological functions, providing with an essential source of cellular fuel and energy storage and are involved in critical signal transduction pathways, such as the inflammation process. Therefore, the metabolomic profiling of the FAs can greatly contribute to the identification of metabolic disruption prior to the onset of symptoms allowing the prediction of

the disease, as well as contribute to the discovery of the disease pathogenesis and proper treatment selection. OAs are intermediate metabolites of critical metabolic pathways, such as the Krebs cycle, carbohydrate metabolism, ketone body metabolism, fatty acid β -oxidation, neurotransmitters turnover and protein metabolism [5]. Therefore, metabolomic analysis of urine OAs may reflect the activity of important metabolic pathways, and can be further used to assess health status, nutritional status, vitamin deficiencies and treatment response [6–9].

There are several metabolomic studies over the last years aiming to discover and evaluate certain metabolic pathways that are shifted or perturbed and can be used as reliable biomarkers for prevention, screening, diagnosis and prognosis based on their ability to predict, diagnose or evaluate the condition of a disease, respectively. These metabolomic studies are based either on untargeted or targeted metabolomics analysis. Untargeted metabolomics is used to detect unknown metabolites and unexpected changes in metabolite concentrations, being able to measure a very large number of metabolites. This approach provides with a relative quantification of the metabolites detected and the biological importance of each metabolite is determined by statistical analysis and biological interpretation [10]. On the other hand, targeted metabolomics aims to analyze a group of specific metabolites that are chemically characterized, predetermined and involved in known metabolic pathways, providing with their accurate identification and quantification [11]. Targeted metabolomics has higher selectivity and sensitivity than the untargeted method and is suitable for the study of certain metabolites/metabolic pathways changes that are already known to be involved in the pathogenesis of a disease.

Selection of experimental procedure for metabolomics analysis

Multiple factors and conditions should be considered during the metabolomic data acquisition and analysis with the aim of biomarker discovery and validation, both in untargeted and targeted metabolomics analysis. These include the choice of instrumentation that should take into account the type of metabolites analyzed, the biological functions of interest, and possible cofounders that should be considered in the analysis. Moreover, patients' characteristics and factors, such as diet and drug interactions, physical activity, age and sex, as well as conditions for sample preparation, processing and storage/preservation are important for the analysis outcome. Finally, of critical importance is the use of quality control samples that account for analytical and biological variations in order to minimize the standard error, as well as the use of internal standards that is crucial for the identification and quantification of the metabolites analyzed [12]. Two of the main techniques

available today for metabolomic data generation are nuclear magnetic resonance (NMR) and mass spectrometry (MS). NMR is based on the energy absorption and re-emission of the atom nuclei due to variations in an external magnetic field in order to produce spectral data [13]. It is a fast and highly reproducible method that can be used for the generation of different types of metabolomic data depending on the atom nuclei being targeted by the applied magnetic field. Hydrogen, however, is the most commonly targeted nucleus (^1H -NMR) in the analysis of biological samples due to its abundance, while other atoms like carbon (^{13}C -NMR) and phosphorus (^{31}P NMR) are less frequently targeted by NMR [14]. NMR can be one- or two- dimensional (1D- or 2D-NMR) and provides with quantification of the concentrations of metabolites and information on their chemical structure [15]. MS is an analytical technique that generates spectral data in the form of a mass-to-charge ratio (m/z) and provides with a relative intensity of the measured compounds after ionization of the biological sample in order to generate peaks signal. The peaks that are generated correspond to the ionized compounds from each molecule detected. There are different MS variants based on different ionization and mass selection methods [16].

MS is usually preceded by a separation step that contributes to the resolution of the complexity of the biological samples and allows the MS analysis of different sets of molecules at different times. The most commonly used separation techniques are Liquid Chromatography (LC) or High-Performance-LC (HPLC) and Gas Chromatography (GC). The technique selection, however, should be done according to the class of metabolites analyzed and in the case of fatty and organic acids GC-MS is the most advantageous technique used for their analysis [17]. GC is generally widely used for qualitative and quantitative sample analysis and can be used in combination with various detectors such as, GC-MS and GC-MS/MS offering very high level of sensitivity and specificity. GC-MS is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC offers a wide spectrum analysis and is used to separate nonpolar, volatile and thermally stable substitutes in a sample, while MS fragments the analyte to be identified on the basis of its mass. Importantly, GC requires the analyte to have significant vapor pressure between 30 and 300°C, and therefore derivatization of the compounds is required to increase their volatility and thermal stability. This process involves derivatizing one or more polar groups on a compound to a less polar group and can be used to increase sensitivity, selectivity, or specificity of the chromatographic separation. The main detection methods for GC-MS are the Electron Ionization (EI) which provides with an untargeted full-scan mass detection and is capable of detecting all, including unknown, compounds in the sample, and Selected Ion Monitoring (SIM) that is a sensitive

targeted approach which allows the identification only of the compounds for which specific acquisition parameters are entered into the analytical method [17].

The advantages of the GC technique compared with other chromatographic methods, such as LC, include an excellent separation spectrum, allowing efficient and precise separation of structurally similar metabolites, improved confidence in sample identification, significantly increased range of thermally labile and low volatility samples and improved sensitivity particularly for compounds that are hard to analyze. Very high specificity is achieved by electron ionization that leads to complex and rich fragmentation patterns which can be exploited to increase the specificity in mass spectral matching. This high level of resolution, specificity and sensitivity that is offered by GC is ideal for analysis of low-polarity volatile metabolites of fats and esters, and high polarity metabolites of amino acids and organic acids converted [18]. GC-MS can also use Time-Of-Flight (TOF) instruments that utilize the times that the ions need to ‘fly’ along an evacuated tube as a means of measuring m/z values and therefore of obtaining a mass spectrum. The ions arrive at the detector in the order of increasing m/z values, so that the ions of the smallest m/z values arrive first, followed by others of increasing m/z value. The main advantages of the TOF technique is that the flight times are so short for all the ions that it is possible for several thousand mass spectra to be accumulated in a very short time frame (even one second). Moreover, acquisition is performed by means of continuous spectra, providing with increased selectivity, high resolution and great sensitivity as there is no ion loss during the separation process. It also allows the use of very small quantities of sample and results in great reproducibility and better signal-to-noise separation. LC or HPLC, on the other hand, can be used to analyze a wide range of low-to-high polarity metabolites, but does not achieve the excellent resolution that is possible with the GC. Moreover, another major advantage of GC-MS compared to LC-MS is the high reproducibility of generated mass spectra using EI, which is a hard ionization that results in the production of very reproducible mass spectra from one instrument to another. These features allow the production of large transferable EI-mass spectral libraries that can be available and accessible to all laboratories, so that each laboratory does not have to develop its own library, a process that can be very costly and time-consuming [18]. However, derivatization even though it can increase the sensitivity and specificity of the analysis, it is also one of the few disadvantages of GC-MS as it can significantly lengthen the time of sample analysis compared to most LC-based methods, while it also alters the compound’s chemical structure, that could potentially misrepresent desired compound after fragmentation in MS. Moreover, it is more suitable for smaller molecular weight compounds, as the ones with high molecular weight are usually insufficiently volatile.

In conclusion, GC-MS offers the most cost-effective, accurate and sensitive way to analyze a variety of endogenous metabolites, particularly fatty acids and organic acids and can be used for both untargeted metabolomic analysis using the full-scan mode that can monitor a range of masses and is useful for identifying unknown compounds, as well as targeted metabolomic analysis using the SIM mode that detects particular metabolites of interest.

Asthma and COPD

Disease background

Asthma is a dynamic, chronic disorder of the lungs, involving airway obstruction caused by inflammation and hyperresponsiveness. It is characterized by spasmodic contraction of airway smooth muscle leading to the classic recurrent and reversible symptoms of wheezing, shortness of breath, cough, and tightness in the chest. Asthma can be often mistaken for chronic obstructive pulmonary disease (COPD) since they share similar symptoms, including coughing, wheezing and shortness of breath. COPD, however, is a general term that describes progressive respiratory diseases like and chronic bronchitis and is characterized by decreased airflow over time, as well as inflammation of the tissues that line the airway. About 40% of people who have COPD also have asthma [19]. Asthma is considered a risk factor for developing COPD and the chance of getting this dual diagnosis increases with age. In that context, several patients are also diagnosed with asthma-COPD overlap, termed as Asthma-COPD overlap syndrome (ACOS), that is a very complex heterogeneous disease without any clear diagnostic or therapeutic guidelines.

Airway obstruction occurs with both diseases but the age of first symptoms is often the distinguishing feature between COPD and asthma. Usually people diagnosed with asthma are children, while COPD symptoms often show up in adults over the age of 40 who are usually current or former smokers. In fact, asthma is the most common pediatric illness affecting more than 6 million children in the United States [1]. Moreover, the causes and the triggers are usually different between the 2 diseases. Although no single cause has been identified as leading to a diagnosis of asthma, several factors in combination have been suggested. It is mostly believed that both genetics and a variety of environmental factors, such as exposure to certain kinds of substances (allergens), that differ from person to person, can trigger asthma. These include pollen, dust mites, mold, pet hair, respiratory infections, physical activity, cold air, smoke, some medications, such as beta blockers and aspirin, stress, sulfites and preservatives added to some foods and beverages such as the monosodium glutamate [20]. On the other hand, smoking and exposure to fumes is a common risk factor for COPD in developing countries leading to 20-30% of regular smokers to develop COPD.

Smoke irritates the lungs, leading the bronchial tubes to lose their natural elasticity and over-expand, leaving air trapped in the lungs while exhaling. Moreover, about 1% of the COPD patients have a genetic disorder that causes deficiency of protein α_1 -antitrypsin which helps in lung protection.

Disease diagnosis

Traditional methods for diagnosis of asthma and COPD are common: the fractional exhaled nitric oxide (FENO) test, that measures the level of nitric oxide in the breath, a sign of inflammation in the lungs, and spirometry, that measures how fast the patient can breathe out and how much air one can hold in his lungs. More specifically, spirometry measures the forced expiratory volume in 1 s (FEV1) as an indicator of airflow limitation (usually FEV1 <60% is predicted for asthma and COPD), as well as the forced expiratory volume in one second/forced vital capacity ratio (FEV1/FVC). Therefore, diagnosis of asthma and COPD requires special markers that can distinguish between the two diseases. The most accessible marker of the Th2 type of inflammation is increased concentrations of blood eosinophils, and consistently, blood and sputum eosinophil counts are significantly higher in patients with asthma compared with COPD. Smoking is considered to be an important factor for the development of COPD but not asthma. It is common that many asthmatic smokers develop also COPD. However, the chronic airflow limitation developed in smoking asthmatics has some particular characteristics that are different from those of COPD in individuals who have never had asthma. In smoking asthmatics with chronic airflow limitation, bronchial hyperresponsiveness, wheezing and allergic rhinitis are more frequent, and they have greater IgE sensitization and higher plasma levels of total IgE compared with COPD developed in non-asthmatic smokers.

Nevertheless, the traditional techniques mentioned above, mainly measuring airways dysfunction and inflammation, are not suitable for all clinical cases, unreliable for efficient prognosis and weak to distinguish between different phenotypes and stages of asthma and COPD [21,22]. Even though there are advances in therapies the last years asthma and COPD have still a significant effect on the health care system. This is due to the complexity and the heterogeneity of both diseases, in respect to their pathophysiologic mechanisms which are several, can interact with each other and may not be present in all patients or at all times. Asthma, in particular, is a heterogeneous syndrome with varying degrees of severity, many clinical classifications based on patient symptoms, lung function, and response to therapy, while COPD has even different phenotypes/subtypes and it is possible that every patient's condition will fluctuate during his or her lifetime [23]. Due to the variety and dynamicity of the pathogenesis of asthma and COPD, the selection of an efficient and personalized therapy based on a single patient profile is complicated. As asthma is associated with episodes of

exacerbations the most commonly used therapy includes inhaled corticosteroids (ICS) that targets airway inflammation in order to maintain and restore asthma control [24]. However, so far, there is no strong link between pathophysiologic characteristics and clinical features or treatment response for COPD and asthma. With the advent of precision medicine and patient-oriented approaches, metabolomics has gained great attention because it provides with a detailed overview of the patient's phenotype. Therefore, it can be used as tool in early diagnosis, before the onset of symptoms, in treatment response monitoring and in targeting the metabolic profile of the disease.

As already mentioned, metabolomics is widely used in research for the identification and validation of specific biomarkers that can be later used in clinical practice for prevention, screening, diagnosis and prognosis of lung diseases, such as asthma and COPD. Discovery of biomarkers is based on the unraveling of pathophysiological mechanisms of lung diseases with the ultimate goal of accurate diagnosis and specialized treatment selection for each asthma and COPD patient (Stockley 2014). The majority of these studies use untargeted metabolomic analysis with the aim to discover asthma-related metabolic markers which will later confirm and evaluate, using targeted analysis on selected pathways and metabolites. The objective of each study can be different depending on the clinical cases used in each study. Some studies aim to identify separate metabolic patterns between asthma patients and healthy controls, other aim to discover metabolic profiles in serum or urine that will allow to discriminate between different levels of severity of asthma (mild, moderate, severe) and clinical parameters [25,26], or distinguish patients by the degree of their lung function [27]. Furthermore, additional studies examined the difference in metabolic profiles between asthma patients with different pathogenic and clinical characteristics, such as obesity [28], or Corticosteroid (CS)-resistant and CS-responsive patients, which would contribute to the selection of personalized treatment and increase the chances of a positive response to that treatment [29]. A set of studies also use Volatile Organic Compounds (VOCs) in order to discriminate between patients and healthy individuals, or stable and unstable episodes of asthma, with the aim to identify a set of exhaled VOCs predictive for asthma [30–32].

Results

Fatty acids in asthma

There is evidence for a wide range of metabolic pathways and metabolites involved, that can serve as biomarkers for asthma and COPD. In particular, there is an emerging role of fatty acids in asthma diagnosis and treatment, as they are associated with asthma pathogenesis and they are found with altered profiles between healthy controls and asthma patients in several studies. Altered

fatty acid metabolic profile is often associated to several asthma clinical characteristics and different levels of asthma severity.

Association of fatty acid metabolic profiles with asthma clinical characteristics and severity

Untargeted metabolomic profiling by LC-MS analysis on plasma samples from 380 children with asthma from the 'Genetic Epidemiology of Asthma in Costa Rica Cohort' [27], identified metabolites associated with three clinical characteristics of asthma severity: (a) airway hyper-responsiveness (AHR) (b) % predicted FEV1/FVC, and (c) FEV1/FVC post-bronchodilator and assessed their discriminatory ability. 15,9% of the metabolites examined were associated with AHR, 17,8% with FEV1/FVC pre-bronchodilator and 27% with FEV1/FVC post-bronchodilator. Common to all three characteristics were linoleic acid, glycerophospholipid and pyrimidine metabolism. The corresponding metabolomic profiles showed significant discriminatory ability, suggesting that there is an asthma severity-specific metabolome which needs to be further examined and evaluated. Moreover, the differences in the metabolomic profiles corresponding to the three pathogenic characteristics indicated that further study and selection of phenotype-specific metabolites is essential for efficient asthma treatment.

Another clinical study was conducted on 76 adults, aiming to first discover different metabolic profiles depending on the severity of asthma, using untargeted LC-MS in serum, and afterwards confirm and evaluate these profiles, using targeted LC-MS [25]. More specifically, 54 patients with asthma were examined (aged 18-70 years), 12 with mild asthma, 20 with moderate asthma, 22 with severe asthma and 22 healthy controls. This study identified 66 metabolites, 15 of which were statistically significantly altered in asthma ($p \leq 0.05$), including fatty acids, such as α -linoleic and oleic acid. In particular, there was a metabolic shift in patients with mild asthma compared to the controls, which was primarily associated with elevated levels of dietary lipids (linoleic acid, oleic acid, α -linolenic acid) and linoleic acid oxidation products. Linoleic acid and its oxidation products are suggested to be contributing to Th2 differentiation in asthma [33], which makes the observation of their excessive abundance an interesting subject for further investigation in larger cohorts. In general, this analysis revealed 2 patterns, a mean difference between controls and patients with mild asthma and a mean difference between patients with severe asthma and the rest of the groups, suggesting that asthma is characterized by a modest metabolic shift in a disease severity-dependent manner. This was further confirmed by a targeted metabolomic analysis on lipid metabolites that integrated metabolomic data with the aim to identify novel genetic and biochemical predictors of asthma control using an integrative “omics” approach [34]. They generated a lipidomic database performing LC-MS on plasma from 20 asthma patients and performed an integrative analysis of

metabolomic, genomic, and methylation data that showed altered metabolic pathways, related to sphingolipid metabolism, in asthma control. The elevated levels of linoleic and oleic acids reported were consistent with the enrichment of linoleic acid metabolism which further underpin the involvement of fatty acids in asthma pathogenesis.

Fatty acids in asthma pathogenesis and nutritional intervention for asthma treatment

As already mentioned NCDs are by far the leading cause of death worldwide due to the increased lifespan and the modern lifestyle in the industrialized world, as well as the change in environmental factors, including the decrease in infectious diseases due to improved hygienic standards, exposure to environmental pollutants and low intake of vitamin D [35]. Main risk factors for NCDs incidence include increased daily caloric intake from processed foods, lack of nutrients, lack of physical activity, obesity and overproduction of adipocytokines, leading to early metabolic pressure [35]. The metabolic hypothesis of many NCDs such as asthma and COPD, has been supported by several researchers stressing the need to find tools that can detect nutritional deficiencies or imbalances [36]. Importantly, concerning the role of fatty acids in asthma pathogenesis, it has been shown that excessive amounts of omega-6 fatty acids lead to a high omega-6 : omega-3 fatty acid ratio and can promote the pathogenesis of chronic diseases in general, including asthma and COPD [37]. Specifically, a ratio of 5: 1 (as in Mediterranean diets) had a beneficial effect on patients with asthma, whereas a ratio of greater than 10: 1 (as in common in Western diets) had adverse consequences [38]. Therefore, an optimal ratio of these two fatty acids may be beneficial with respect to asthma symptoms.

The clinical importance of measuring FA dietary intake is associated with their essential roles in inflammation [39]. Therefore, the aim of several studies the last 15 years was to assess whether a higher intake of omega-6 or a lower intake of omega-3 fatty acids could increase the risk of asthma. In order to study the effect of food or dietary supplements intake on asthma symptoms, the great majority of the studies used food frequency questionnaires to estimate food intake and measurement of several biomarkers related to asthma symptoms, such as FEV1, FeNO, as well as self-reported asthma symptoms (e.g. wheeze). An example is a 20 years follow-up longitudinal analysis that was conducted in a cohort of 4162 Americans (aged 18-30 years) diagnosed with asthma at baseline in 1985 [40]. Dietary intake was validated using food-frequency questionnaires in 1985, 1992, and 2005. During this study 446 incident cases of asthma were identified between 1985 and 2005. High intake of n-3 polyunsaturated fatty acids (n-3 PUFA) was significantly associated with low incidence of asthma after adjustment for demographic, lifestyle, and dietary factors, and especially Docosahexaenoic acid (DHA) that appeared to have a more significant effect

than Eicosapentaenoic acid (EPA). These results suggested that high intakes of n-3 PUFA are longitudinally associated with low incidence of asthma. In agreement with this, McKeever et al., investigated the relation between individual fatty acid intakes and lung function by measuring FEV1 and self-reported wheeze, asthma and COPD symptoms in a sample of more than 13,000 Dutch adults [41]. High intake of n-6 polyunsaturated fatty acids (n-6 PUFA) was associated with significant reduction in FEV1, especially in smokers. Moreover, a number of studies introduced a protective effect of n-3 PUFA and α -linolenic acid (ALA) in asthma control. A study on 174 asthmatics (mean age 40 years) investigated the association between dietary intakes with FEV1 and FeNO [42]. High intakes of n-3 PUFA, ALA and SFA were associated with low FeNO and good asthma control, while high n-6: n-3 PUFA ratio increased the risk for uncontrolled asthma after adjusting for energy intake, sex, age, education and use of inhaled corticosteroids.

Nevertheless, the great majority of the studies estimated food intake by using food frequency questionnaires, which are usually not reliable due to recall bias and lack of memory of the patients or their parents in the case of children-based studies. However, some studies also used biochemical measurement of actual FA levels for estimation of food intake, which, unlike dietary frequency questionnaires, enables the accurate estimation of both FA dietary intake and endogenous FA synthesis [43]. An example is a study that measured the erythrocyte membrane fatty acids by MS [44]. Higher levels of erythrocyte membrane linoleic acid were associated with a lower risk of asthma. Adams et al., also highlighted the potential protective role of n-3 PUFA in asthma, aiming to determine the association between seafood intake, composition of PUFA in the serum and clinical symptoms of asthma in adults [45]. They used respiratory health survey questionnaire, spirometry, skin prick tests and methacholine challenge tests, while the levels of n-3 and n-6 PUFA were measured in the serum of the 642 participants. The subjects were mainly female and current smokers and they consumed mostly fish and less seafood. They showed that in adjusted models the increased levels of EPA and Docosapentaenoic acid (DPA), as well as total n-3 PUFA serum composition were significantly associated with a decreased risk of non-specific bronchial hyperresponsiveness (NSBH), while total n-6 PUFA was associated with an increased risk of NSBH.

In accordance with the above, several studies supported that adherence to the Mediterranean diet that is rich in monounsaturated fatty acids, a balanced ratio of n-6 : n-3 essential fatty acids and high amounts of fiber and antioxidants, such as vitamins E and C, resveratrol, polyphenols, selenium, glutathione, can promote good health [46] and is further associated with improved asthma control [47]. The effect of Mediterranean diet and specifically fatty fish intake on asthma was studied by a very recent intervention study that was conducted on 64 children with mild asthma (52% male and 48% female) [48]. Thirty-three children were in the control group and 31 in the intervention group,

whose fatty fish intake was increased significantly during the 6-month period. The effect of the intervention was evident only after adjustment for age, sex, body mass index and regular physical activity. Even though, there was no significant effect on spirometry, asthma control and quality of life scores after fatty fish intake, that can be explained by the normal lung function, and well-controlled asthma of the children participating in the study, some recent meta-analysis documented that regular fish intake can be beneficial for asthma control and the amelioration of its symptoms. In particular, the meta-analysis showed that regular fish intake (≥ 1 /week) reduced ‘current asthma’ and ‘current wheeze’ in children aged 0-4.5 years, whereas fatty fish intake reduced ‘current asthma’ in 8-14 year old children with asthma [47]. These results indicated a direct effect of fatty fish intake on childhood asthma not only based on questionnaires, but also by measuring plasma fatty acid composition that is a much more reliable marker of dietary fat intake in children [49].

Organic acids in asthma

Organic acids and energy metabolism, including Krebs cycle metabolites, have also been suggested as potential biomarkers for discrimination among different pathogenic backgrounds and clinical characteristics of asthma patients. Several clinical studies the past few years aimed to distinguish among different metabolic profiles corresponding to divergent pathogenic phenotypes and clinical parameters of asthma, as well as responses to different treatments.

Metabolic profiling of patients with divergent pathogenic background

A study comparing the metabolomic profiles of exhaled breath condensate (EBC) from obese asthmatic (OA), lean asthmatic (LA) and obese non-asthmatic (ONA) patients, aimed to identify biomarkers specific for an “asthmatic-obese” metabolic phenotype [28]. In this study, the profiles of 25 OA patients, 30 ONA subjects, and 30 mild-to-moderate LA age-matched patients were analyzed by NMR and validated. Strong regression models distinguished OA patients from ONA subjects, as well as OA patients from LA patients, while specific biomarkers for class separation were identified, including metabolites involved in the methane, pyruvate, glyoxylate and dicarboxylate metabolic pathways. These results indicated that OA patients present a respiratory metabolic fingerprint very different from that of patients affected by asthma or obesity independently, suggesting special pathophysiologic pathways involved in the pathogenesis of asthma in adult obese patients. This unique metabolomic pattern for obese asthmatic patients could contribute to a more accurate treatment selection for this kind of patients.

More recent metabolomic studies aimed to explore the pathogenesis of asthma based on its heterogeneity and distinguish different metabolic profiles corresponding to different asthma

phenotypes. Patients with different pathogenic profiles were included in a study performing untargeted metabolomic analysis in serum [50]. These phenotypes included eosinophilic asthmatics (EA, n=13), non-eosinophilic asthmatics (NEA, n=16), and healthy controls (HC, n=15). They used Ultra Performance Liquid Chromatography–Mass Spectrometry technique to perform the metabolomic analysis which showed distinction between the different phenotypes EA, NEA, and HC. Eighteen different metabolites were recognized between the three groups which were involved in 10 perturbed metabolic pathways, with Glycerophospholipid metabolism, retinol metabolism, and sphingolipid metabolism as the most significant perturbed three pathways between the phenotypes. These results indicate immune regulation, nutrients and energy metabolism are involved in divergent inflammatory phenotypes of asthma, that could contribute to selection of optimal therapeutic strategy for each heterogenic asthma phenotype.

Metabolic profiling of asthma patients based on different clinical parameters

Another study examined the relationship between oxidative stress and clinical characteristics, such as lung function, eosinophilic inflammation and disease severity in asthmatic patients [26]. Targeted urinary metabolomic analysis was performed using aliphatic aldehydes and alkanes as targets, by solid-phase microextraction (SPME) followed by a high-resolution GC-TOF-MS on fifty-seven asthmatic patients (mean age 45 years), including 17 obese. The aim of the study was to investigate the link between lipid peroxidation and the clinical characteristics of nonobese asthmatics, such as disease severity, lung function, and eosinophilic inflammation. 34 aliphatic alkanes and aldehydes were used to correlate lipid peroxidation urinary metabolomic profile with several clinical parameters of asthma, including control scores (asthma control test (ACT)), severity scores (severity of asthma score (SOA)), lung function (FEV1 (%), FEV25–75 (%)), and Th2 inflammatory biomarkers (FeNO, blood eosinophils (%), and serum IgE (log UI)). The analysis was carried out excluding obese patients as obesity is associated with an increased oxidative stress and systemic inflammation, being a potential mechanism for the increasing asthma severity. Based on the results significant models were obtained for several clinical parameters such as SOA, FEV1, FeNO, blood eosinophils, and serum IgE, suggesting that there is a correlation between metabolic profiles and clinical characteristics of asthma that could be used in diagnosis and personalized treatment.

Another very recent study aimed to correlate urinary organic acids with pulmonary diagnostic tests and asthma control in Greek asthmatic children [51]. Seventy-two asthmatic children (5-12 years old) were recruited from a pediatric asthma clinic in Athens, Greece. Spirometry and exhaled nitric oxide analysis were used to assess pulmonary function, while asthma control was measured using the Asthma Control Questionnaire. Targeted metabolomic analysis of 34 urinary organic acids was

conducted by GC-MS, that showed a statistically significant difference between girls and boys for asthma control ($P = 0.02$) and lactic acid ($P = 0.03$). Some of the statistically significant correlations found, included the ones between lactic acid and FEV1-FVC, 4- hydroxyphenylacetic acid and FEV1-FVC, 5-hydroxyindoleacetic acid and FEV1/FVC-FeNO, as well as glycolic acid with Peak Expiratory Flow (PEF) and malic acid with asthma control. So, this is another example of correlations between metabolites and clinical parameters that can be used in the detection of novel biomarkers for asthma monitoring and therapeutic targets for childhood asthma.

Metabolic profiling of patients receiving corticosteroid treatment

As mentioned above, treatment of asthmatic patients with inhaled corticosteroids (CS) is the most commonly used treatment in asthma. A set of metabolomic studies aimed to analyze the response to CS-treatment of asthma patients with different pathogenic profiles. An example is a study aiming to further understand the CS-resistance among children with severe asthma [29]. The metabolomic profiles of urine samples from 15 CS-responsive and 15 CS-nonresponsive children were analyzed to determine potential biomarkers related to CS resistance. The analysis determined 30 metabolites showing significantly different levels between CS responders and CS non-responders, including 5 metabolites that are involved in Tyrosine metabolism, degradation of aromatic compounds, and glutathione metabolism. These metabolites could be used as potential biomarkers related to CS-resistant children with severe asthma and could contribute after being evaluated, to the further understanding and thus better selection of the right treatment for these patients.

Moreover, concerning the CS treatment in asthma, inhaled budesonide and salbutamol represent the most important and frequently used glucocorticoids as drugs for asthmatic children during acute exacerbation. In order to examine the metabolomic profiles of the 2 drugs a study was conducted on 69 children with asthma during acute exacerbation [52]. Their serum and urine were analyzed using high-resolution NMR. The distinct metabolic profiles obtained changes after inhaled budesonide and salbutamol in asthmatic children during acute exacerbation were identified and analyzed. The metabolites with different profiles were 22 in the serum and 21 in the urine involved in seven metabolic pathways: citrate cycle, methane metabolism, pyruvate metabolism, glyoxylate/dicarboxylate metabolism, taurine and hypotaurine metabolism, as well as, amino-acid metabolism including arginine and proline metabolism, and glycine, serine and threonine metabolism as the most significantly affected pathways. These results suggested significant alteration of metabolic profiles after combined treatment with inhaled budesonide and salbutamol in asthmatic children that could lead to airway dysfunction through the underlying mechanism of epithelial damage and airway smooth muscle proliferation [53], or increased risk of insulin

resistance, metabolic syndrome, diabetes and childhood obesity [54]. Therefore, even though many children during asthma exacerbation are prescribed with glucocorticoids their metabolic effect should not be neglected.

Longitudinal metabolic profiling for asthma control

Asthma control has not yet reached a high level that satisfies the guidelines for asthma management. Therefore, non-invasive longitudinal monitoring of airway inflammation may help to improve the level of asthma control. A study focused on the longitudinal analysis of the dynamics of metabolites contributing to asthma development was conducted on thirty children with asthma and paired healthy controls from a prospective birth cohort [55]. Urinary samples were collected at ages 1, 2, 3, and 4 years and analyzed by NMR coupled with partial least squares discriminant analysis (PLS-DA). From the longitudinal analysis of 172 urine samples in total, 4 metabolites were identified significantly associated with childhood asthma development, including dimethylamine, a metabolite produced by intestinal bacteria and 1-methylnicotinamide and allantoin that were found persistently lower in children with asthma, with a peak difference at age 3 years. Furthermore, a significant inverse correlation was found between allantoin and house dust mite sensitization. These results suggested that longitudinal urinary metabolomic profiling could provide a link between microbe-environment and the development of childhood asthma, while 1-methylnicotinamide and allantoin could be used as potential specific biomarkers for asthma as they are possibly associated with allergic reactions triggered by exposures to allergens.

Metabolomic profiling of VOCs in exhaled breath condensate (EBC)

Exhaled breath analysis is a potential non-invasive tool for diagnosing and monitoring airway diseases including asthma. GC-MS and electrochemical sensor arrays are the main techniques to detect VOCs in exhaled breath. Importantly, exhaled volatile metabolites could potentially identify longitudinal changes between clinically different asthma episodes. In these lines, a metabolomic study measured VOCs by GC/MS and electronic nose (eNose) technology in order to discriminate between clinically stable and unstable episodes of asthma [32]. Twenty-three with partly controlled mild to moderate persistent asthma using CS treatment were included in this study. Exhaled metabolites were measured at baseline, during loss of control and after recovery in order to examine the metabolic profiles corresponding to cases controlled by CS-treatment and the ones after pausing CS-treatment. Finally, associations between exhaled metabolites and sputum inflammation markers were examined in order to further evaluate the metabolic profiles identified. Analysis of VOCs by eNose showed 95% correct classification for baseline vs loss of control and 86% for loss of control vs recovery. GC/MS analysis showed lower accuracy of 68% for baseline vs loss of control and 77%

for loss of control vs recovery, while significant association between exhaled metabolites identified by GC/MS and sputum eosinophils was shown ($P < 0.01$). These results indicate that metabolomic analysis of VOCs by GC/MS and especially eNose and their further comparison with sputum eosinophils could identify biomarkers important for discrimination between clinically stable episodes and loss of asthma control.

Carraro et al., also used VOCs metabolomic analysis in order to discriminate between different asthma phenotypes, mostly focused on severe asthma in children [56]. Forty-two asthmatic children (8-17 years old), from whom 31 had non-severe asthma phenotype, while 11 had severe asthma phenotype, and 15 healthy children as controls, participated in this study. The participants performed exhaled nitric oxide measurement, spirometry and EBC collection and the samples were analyzed by MS. The metabolomic analysis indicated that each group of children is characterized by a different metabolic profile, suggesting that EBC could be used to fully discriminate between asthma phenotypes of different severity. More specifically, metabolites that appeared with different profiles between the 3 groups, healthy controls, mild asthma and severe asthma, were retinoic acid, adenosine and vitamin D.

Early wheeze is common in preschool children but the underlying pathophysiology is still under investigation. VOCs in EBC can serve as non-invasive markers of early wheeze. Van de Kant et al aimed to study whether a VOC profile can distinguish between children with and without recurrent wheeze [57]. Two hundred and two children with and fifty children without recurrent wheeze were included in the study and exhaled VOCs were analyzed by GC-TOF-MS. In total, 913 different VOCs were detected and after adjusting for age and sex they ended up with a model based on 28 VOCs, including acetophenone; 2-propen-1-ol; 1,2,3-trimethylbenzene; pentanoic acid; nitrocyclohexane; 4-methyl-1-decene; 3-methyl-1-butene; hexadecan-2-ol and naphthalene. This model could correctly classify 83% of the children (84% sensitivity and 80% specificity) and 73% after a 6-fold cross-validation, suggesting that VOCs in EBC can serve as biomarkers for the detection of an early asthma phenotype such as the recurrent wheeze, potentially contributing to asthma prevention or early diagnosis.

More studies analyzed VOCs aiming to the identification and evaluation of biomarkers for asthma monitoring and control. More specifically, a study used FeNO, VOCs and asthma control in order to explore the association between inflammatory markers in exhaled breath that could potentially discriminate between children with persistently controlled and uncontrolled asthma [30]. Ninety-six asthmatic children were followed-up in a one-year observational study. Every 2 months, the following parameters were assessed: asthma control, FeNO, lung function (FEV1) and forced vital

capacity (FVC), exhaled VOCs, and cytokines/chemokines in exhaled breath condensate (EBC). No significant association was found between the exhaled inflammatory markers (FeNO, markers in EBC, VOCs) and asthma control. However, 15 exhaled VOCs could discriminate between subgroups of children with persistently controlled and uncontrolled asthma during all clinical visits.

Based on the results of the previous study a similar one-year prospective observational study on the same 96 asthmatic children aimed to identify a more predictive metabolomic model for asthma exacerbation in children and examine the identity of predictive biomarkers [31]. The patients visited every 2 months for asthma control during which FeNO, lung function (FEV1, FEV1/VC) and VOCs in exhaled breath were determined by gas chromatography time-of-flight mass spectrometry. The sensitivity of the predictive model for exacerbations 14 days after sampling was 88% and 21 days after sampling was 63%. Importantly, the predictive power of a set of VOCs had an inverse relationship with the time between sampling of exhaled breath and the onset of exacerbation. The VOCs selected for the classification model were 7: 3 aldehydes: 2-ethylhexanal, octanal and nonanal, a ketone 6, 10-dimethyl-5, 9-undecadien-2-one, an aromatic compound 2-methylfuran or 3-methylfuran, a hydrocarbon 1, 2-dimethylcyclohexane and one unidentified VOC. These results indicated that prediction of asthma exacerbations 14 days after sampling is significantly accurate and reliable and could be used for clinical application. The use of a validated model to predict asthma exacerbations based on identified metabolites could be crucial for efficient treatment and control of divergent clinical cases of asthma.

Metabolomic profiling for asthma and COPD discrimination

The metabolomic analysis of COPD patients with different pathogenic background the recent years has revealed metabolic dysregulation appointing as potential biomarkers for the disease metabolites involved in amino-acid metabolism, lipid metabolism, energy metabolism, as well as oxidative stress [58–61]. For example, alterations in the sphingolipid metabolism [60] and dysregulation of lipid metabolism during the onset of COPD [59,61]. Moreover, additional studies have showed perturbed synthesis of membrane phospholipids further supporting a hypothesis of fatty acids oxidation and tryptophan metabolism dysregulation leading to increased oxidative stress [62]. In addition to these results smoke exposure and reduced levels of free carnitine in the lung can also lead to progressive emphysema and COPD due to dysregulation of FAs. A possible mechanism for the increased oxidative process is that fatty acid levels in cytosol are increased due to smoke exposure and impaired carnitine metabolism leading to lipotoxicity [58].

Importantly, other metabolic pathways that appear to be altered in COPD are involved in amino-acid metabolism. A great number of amino-acids seems to be consistently affected in COPD among

the clinical studies, an observation that could be associated to the weight loss, indicating hypermetabolism, that is one of the crucial clinical symptoms of COPD. There is a general amino acid metabolic pattern in COPD that includes a reduced plasma BCAAs level, and a decreased muscle glutamate concentration. Alterations in BCAAs metabolism appear to be influenced by the degree of muscle wasting. For example, low ratio of BCAAs to aromatic amino acids was significantly correlated with percentage of ideal body weight, percentage of arm-muscle circumference and % FEV1 potentially related to hypermetabolism and respiratory muscle weakness [63]. The reduction in glutamate status is linked to reduced muscle glutathione levels and enhanced glycolysis which is evident from the increase in plasma lactate during exercise in COPD patients [64,65].

Some studies were focused on examining and comparing the metabolic profiles of adults with asthma and adults with COPD. Urine samples from adults with asthma and COPD before and after an exacerbation and from adults with stable asthma and COPD were collected and analyzed by NMR [59]. Eighty-six metabolites were measured per sample in order to create predictive models of separation. After analysis some metabolites were found different between patients with asthma and patients with COPD both during exacerbation, as well as in follow-up after exacerbation. These metabolites included 3-hydroxyisovalerate, arginine, ascorbate, choline, citrate, creatinine, dimethylamine, betaine, guanidinoacetate, glucose, glutamine, glycine, glycolate, histidine, hypoxanthine, isoleucine, methanol, pantothenate, urea, succinate, xylose, taurine, and 1-methylnicotinamide, several of them involved in amino-acid and energy metabolism. The predictive model used 91 patients with asthma and 38 with COPD, and after removal of irrelevant metabolites it had more than 90% accuracy to correctly classify blinded asthmatic patients, suggesting that metabolomic analysis can be useful to discriminate between asthma and COPD cases.

ACO is, as already mentioned, a very complex and heterogeneous disease that its existence based on its pathophysiology as a unique disease entity is still unclear. Patients with ACO have a faster lung function decline, more frequent exacerbations, and worse quality of life than those with COPD or asthma alone. The aim of a study was to examine whether ACO has distinct metabolic profile from asthma and COPD [66]. Serum samples from patients with moderate and severe asthma (based on the Global Initiative for Asthma (GINA) guidelines), patients with moderate and severe COPD (based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines) and ACO patients (diagnosed by joint GOLD and GINA guidelines) and healthy controls were analyzed by NMR. Metabolomic analysis showed that 12 metabolites including lipids, amino-acids such as isoleucine, valine, glutamate, L-leucine, lysine, asparagine, phenylalanine, N-acetylglycoproteins (NAG), citric acid and glucose were perturbed in ACO patients compared with asthma and COPD

patients. These metabolites were further validated suggesting that ACO patients may have some of their metabolic pathways heavily disturbed, including energy and amino-acid metabolism compared to asthma and COPD, triggering the further unraveling of the pathophysiological complexities associated with the disease.

Discussion

Asthma is a chronic disease with various symptoms of the respiratory tract but also divergent metabolic fingerprint. As already mentioned, the metabolic pressure is prominent in several chronic diseases, including asthma and COPD, raising the scientific interest on metabolomics as an important part of the study of such diseases. Asthma and COPD metabolomics studies to date report a number of replicated biologically plausible metabolites and metabolomic pathways associated with the development and manifestation of asthma and COPD. More specifically, untargeted metabolomic profiling of asthma and COPD patients has indicated several potential metabolites and metabolic pathways as biomarkers that, although diverse, can be broadly categorized based on their physiological or molecular roles in: 1) immune response, signaling, and inflammation, 2) amino-acid metabolism, 3) lipid metabolism, 4) energy metabolism, as well as 5) oxidative stress and hypoxia [27].

Fatty acid metabolomic profiling is of high importance for asthma and COPD, as FAs play an essential role in the development and resolution of inflammatory pathways relevant to the pathophysiology of asthma and COPD. Moreover, there is an enrichment of pathways reflecting increased metabolism of lipids, steroids, and bile acids that are fundamental to asthma pathogenesis. Organic acid metabolism is also often found altered in asthma and COPD, as such patients often have some airway obstruction, which could cause a mild degree of hypoxemic stress on body tissues. An organic acid found with altered metabolic profile in asthmatic patients in many studies is trans-aconitate [67,68]. Also, other metabolites related to the citric acid cycle, such as 2-oxaloglutarate, succinate, fumarate, 3-hydroxy-3-methylglutarate and cis-aconitate, seem to be critical in asthma patient stratification [67]. Moreover, metabolites participating in energy metabolism, such as 2-hydroxyisobutyrate, 3-hydroxybutyrate, and 3-methyladipate that are known for their roles in glucose and lipid metabolism, are found altered in asthma [69]. Similarly, levels of metabolites participating in the tricarboxylic acid cycle and in cellular energy metabolism in the lungs are perturbed in asthma [70]. It is possible that alterations in these pathways may reflect the reduced ability of the damaged lung to fulfill the energy demands of activated inflammatory cells in the allergic airway [27].

Amino acid metabolism is also found highly altered in most metabolomics studies on asthma and COPD. Amino acids are mediators of immunological activities in asthma and have also antioxidant functions, such as taurine, glycine, glutamine, and glutamate that may have protective effects [27]. Moreover, levels of glutamine, phenylalanine, 3-methylhistidine, BCAAs, glycine, aspartate, arginine, alanine, cysteine, ornithine are consistently affected in COPD patients compared to healthy controls. The most consistent metabolic pattern of amino acids in asthma and COPD includes reduced plasma levels of the BCAAs leucine, isoleucine and valine, and a decreased muscle glutamate concentration [64,65]. The reduction in glutamate status was linked to reduced muscle glutathione levels and enhanced glycolysis, reflected by the increase in plasma lactate during exercise in COPD patients [64,65]. When lactate is elevated during anaerobic exercise alanine levels are expected to rise as part of gluconeogenesis, while acetone is formed when stores of glucose are low and stores of oxaloacetate have been exhausted [71].

Downregulation of circulating BCAAs [72,73], is thought to be a hallmark of cachexia. Therefore, these observed patterns including another common COPD-associated metabolic pattern with altered plasma levels of alanine, tyrosine, glutamine, aspartic acid, lysine, proline, and ornithine, may be associated with the cachexia and low body weight present in many patients with asthma and COPD. These patients exhibit increased Resting Energy Expenditure (REE) and are in a state of hypermetabolism, that is the increased consumption of calories per kilogram, to cover the increased effort for respiration [74–76]. It is suggested that disturbed amino acid metabolism is a characteristic of underweight asthma and COPD patients and may be related to disease severity and reduced respiratory muscle efficiency. This is further supported by the finding that low levels of BCAAs and increased REE were significantly correlated with the percentage of ideal body weight, percentage of arm-muscle circumference and % FEV1, especially in underweight COPD patients [63]. Ubhi et al. observed a slightly significant increase in plasma amino acids of cachectic compared to non-cachectic COPD patients [73], while other cachectic clinical studies exhibited lower levels of circulating amino acids during cachexia [77]. The diverse analysis of all these altered metabolic pathways can give great insight into a patient's biochemical individuality, leading to more targeted therapeutic recommendations.

In summary there are some common patterns of metabolic alterations caused by asthma and COPD, however, there is some variability and inconsistency between the results of different metabolomic studies that hampers the establishment of a combination of validated biomarkers for asthma and COPD. Much of this heterogeneity stems from lack of standardization in the field, different sample preparation, handling and processing conditions, different quality control samples and internal standards, different number of replicates used in each study that are rarely mentioned, different

characteristics of the subjects involved, including age, sex, physical activity, nutritional status, their exposure to different environmental conditions and very importantly the heterogeneity of asthma and COPD with respect to the lung function and its pathogenesis [12,78].

For the limitation of heterogeneity and inconsistency, the thorough examination and better understanding of the results, it is important to integrate untargeted metabolomic data from two different sample specimens (e.g. EBC and serum) with targeted metabolomic analysis of one of them (e.g. serum) and associate them with demographic and clinical characteristics. Combination of untargeted, targeted metabolomic data and clinical/demographic data can be a strong tool to discriminate between different subtypes of the disease, early diagnosis, prevention and prediction of treatment response [60]. Along these lines EBC is an optimal biospecimen, a noninvasive method approach for collecting samples with more direct relevance to the organ of interest, which however, is not often used in the metabolomics studies, where blood and urine prevail. Another important issue is the role of the biomarkers identified and evaluated for asthma and COPD. Many studies focus on distinguishing asthma cases from healthy patients, even though clinical markers and criteria for asthma diagnosis already exist. Therefore, it would be more informative and useful to perform more studies focusing on the discrimination of different subtypes, which are currently not well-defined, or on asthma prediction that is of great clinical value. Only a few studies aimed to identify predictive biomarkers, for example by focusing on wheezing in preschoolers, which could be considered an early asthma phenotype [57].

Moreover, metabolomics could be used as an assessment tool of the nutritional needs of patients with asthma and COPD, as nutritional deficiency plays a key role in these patients, partially explaining the metabolic imbalances observed in asthma and COPD [79]. As a result, nutritional intervention based on metabolomics analysis can be extremely beneficial for asthma and COPD, such as intake of omega-3 polyunsaturated fatty acids that has been shown to have anti-inflammatory effect in asthma and COPD [80] and intake of Vitamin D that has an immune-modulatory effect, important for the improvement of muscle weakness and exacerbation events in COPD patients [81,82].

Since the ultimate goal is the clinical translation of the data deriving from the metabolomic analysis, a critical issue is the determination of specificity of the identified biomarkers. The majority of biomarkers reflect a general biological profile of a dysregulated and disturbed physiological state, instead of being specific to the asthma phenotype. Many studies show that the VOCs profile was similar to many different asthma phenotypes [31,57], while several distinct respiratory disorders may present similar metabolic profiles with asthma, such as ARDS or exposure to environmental

pollution [83], making it very hard to determine their specificity to asthma. Finally, some metabolites altered in asthma, especially the amino acids, have been also associated with other chronic diseases including multiple malignancies [84], psoriasis, thyroid disorders and others, limiting their utility as asthma-specific biomarkers, even though they can be highly involved in asthma pathogenesis.

Psoriasis

Disease background and types

Psoriasis is a common, chronic, inflammatory skin disease which according to the global report on psoriasis by the World Health Organization affects approximately 100 million individuals around the globe or 2-3% of the total population (Global Report on Psoriasis 2016, WHO). The prevalence rates can also reach 11% in Caucasian and Scandinavian populations [85–88].

Although the underlying cause and pathogenesis are still under investigation, it is known that genetic predisposition combined with environmental modifiable factors including obesity, tobacco, psychological stress and alcohol can lead to the development of the diseases [89]. The effect of genetics on the immune responses in psoriasis should be also determined, as the genetic variants associated with psoriasis have been shown to be involved in different biological processes, including antigen presentation, inflammation, and keratinocyte biology [90]. Although, there is growing evidence that psoriasis should be considered as an autoimmune disease including the genetic background and the biochemical disruption that is similar to other autoimmune diseases, it is an ongoing debate given that no auto-antigen has been conclusively discovered that triggers the disease and no self-reactive T cells have been identified [91]. Psoriasis is characterized by Increased proliferation of keratinocytes and endothelial cells in conjunction with inflammation leading to the distinct epidermal and vascular hyperplasia. The lesional psoriatic skin is red, itchy, dry and patchy which confers significant physical and psychological distress and impairment in Psoriasis patients. Psoriasis is a chronic disease that has no permanent cure but its symptoms can be managed and put under control. The main goal of any treatment, topical or systemic, is to stop the fast growth of the skin cells and limit the inflammation. The inflammatory pathways that are active in psoriasis overlap between the different types of the disease, but also show significant differences corresponding to different phenotype and treatment outcomes.

There are five types of psoriasis depending on its dermatologic manifestations, with the most prevalent being psoriasis vulgaris, also called plaque-type psoriasis, which affects 85-90% of patients with psoriasis. Psoriasis is strongly related to other comorbidities including inflammatory

bowel disease, obesity, atherosclerosis, diabetes and metabolic syndrome [92]. Also, osteoporosis, and COPD has also been reported to co-exist with psoriasis [93].

Notably, people with psoriasis are predominantly male, elderly and smokers, with elevated blood pressure, blood glucose and hypertriglyceridemia values compared to obese people without psoriasis [94–96]. Another metabolic factor related to psoriasis is insulin resistance, which causes chronic inflammation that favors the occurrence of psoriasis and other inflammatory conditions. According to studies, patients with psoriasis have an increased risk of developing type 2 diabetes [97–99]. Psoriasis has been also shown to be associated with lack and resistance to vitamin D, and treatment with vitamin D3 has been proven beneficial for psoriasis treatment [100,101]. Moreover, another comorbidity is psoriatic arthritis that is a distinct form of psoriasis characterized by the coexistence of clinical manifestations of arthritis and occurs in up to 25% of patients with psoriasis. It causes inflammation in the joints, mostly localized on the wrists, knees, ankles and neck. Overall, several clinical features of psoriasis suggest that may be associated with metabolic changes that could be important to determine the disease pathogenesis and treatment response

Therefore, metabolomics is, an ideal approach for investigating the link between psoriasis, cardiometabolic comorbidities, and the microbiome, as it can capture the downstream effects of environmental factors, such as diet and lifestyle on psoriasis, allowing for the identification of novel biomarkers that aid in the prediction, diagnosis, and understanding of the pathogenesis of the disease.

Fatty Acids in Psoriasis

Fatty acid profiling for Psoriasis diagnosis

The metabolomic analysis of fatty acids in serum has started several decades ago but its progress is very slow with only a few studies focused on the role of fatty acids in psoriasis the last years. Several studies have been focused on the serum metabolic differences between healthy individuals and psoriasis vulgaris patients, aiming to discover potential biomarkers for prevention, diagnosis, identification of different psoriatic phenotypes, treatment response and unraveling of pathogenesis of psoriasis.

An untargeted high-throughput metabolomics analysis based on LC-MS, was applied to study the serum metabolic profiles of 150 individuals, 75 psoriasis patients and 75 healthy controls [102]. This analysis identified 44 potential biomarkers mainly involved in glycerophospholipid metabolism, sphingolipid metabolism, arachidonic acid metabolism, linoleic acid metabolism and bile acid biosynthesis, suggesting a role of lipid and fatty acid metabolism in psoriasis. Moreover, another

study aimed to identify the low-molecular weight compounds contributing to the metabolomic profiling of psoriasis and to provide computational models that would help with the classification and monitoring of the severity of the disease [103]. For this reason, they performed both untargeted and targeted metabolomic analysis. For the untargeted analysis they used 40 volunteers, 20 diagnosed with plaque psoriasis and 20 age and sex-matched controls (aged 20-75 years). For the targeted analysis, the number of individuals involved was much higher, 106 volunteers, 55 patients with psoriasis and 51 controls (aged 20-75 years). They compared the results of the untargeted and the targeted analysis in order to find differences between the metabolic profiles of the patients compared to the healthy controls. The main differences were found in the concentrations of acylcarnitines, phosphatidylcholines, amino acids, urea, phytol, and 1,11-undecanedicarboxylic acid. The data from the targeted analysis were used to build classification models for psoriasis and reached 77% sensitivity and 74% specificity.

Additional studies focused on unraveling psoriasis pathogenesis sought out to determine serum metabolomic profiles among patients with psoriasis and healthy controls in order to identify psoriasis biomarkers. Kang et al. analyzed by GC-MS the serum metabolic profiles of 29 individuals, 14 patients with psoriasis and 15 sex- and age- matched healthy controls [104]. Statistical analysis of these profiles indicated perturbed serum metabolites in the patient group compared to the healthy controls. More specifically, psoriasis patients had (i) increased amino acid levels including asparagine, aspartic acid, isoleucine, phenylalanine, ornithine and proline, (ii) elevated glycolytic activity such as increased lactic acid; (iii) increased urea cycle activity and (iv) decreased fatty acid syntheses including decreased levels of crotonic acid and azelaic acid, all compared to healthy controls. These results suggested increased amino acid levels, urine cycle activity and glycolysis pathway in psoriasis consistent with previous studies [105,106]. The increased metabolites in these pathways may well explain the observed keratinocyte hyperproliferation and elevated proteolysis activity in patients with psoriasis resulting in increased demand for psoriasis-enriched protein biosynthesis. Glycolytic activity was also significantly increased as reflected by high lactic acid levels in patients with psoriasis. This could be a result of host response to inflammation induced in psoriasis, a phenotype found in wound healing [107]. Moreover, the analysis revealed significantly lower levels of crotonic acid and azelaic acid in patients with psoriasis compared with healthy individuals, that is consistent with the fact that azelaic acid topical treatment has therapeutic efficacy in patients with psoriasis vulgaris [108]. All these data collectively contribute to the elucidation of the pathogenesis of psoriasis.

Metabolomic analysis can also be performed on epidermis lesions for the study of psoriasis. Hammarstrom et al. performed a targeted metabolomic analysis measuring the concentrations of

prostaglandins E2 and F2alpha, free arachidonic acid, and 12L-hydroxy-5,8,10,14-eicosatetraenoic acid in specimens of uninvolved and involved epidermis of psoriasis patients by deuterium-labeled carriers and multiple ion analysis [109]. The analysis revealed a strong correlation between arachidonic acid and hydroxyeicosatetraenoic acid levels in involved epidermis and significantly increased levels of arachidonic acid and 12L-hydroxy-5,8,10,14-eicosatetraenoic acid in involved epidermis compared to the uninvolved. Similar results showed another study that analyzed human skin samples coming from the collection of exudates from abraded sites, a suitable method for psoriatic skin sampling [110]. As in the Hammarstrom et al., this analysis revealed that arachidonic acid and 12-monohydroxyeicosatetraenoic acid, but not prostaglandin E2, were significantly increased in exudate from abraded psoriatic skin lesions compared to uninvolved skin. The authors suggested that fatty acid metabolic profiles are altered in psoriasis and can be used for the identification of diagnostic and pathogenetic biomarkers of psoriasis.

Fatty acid profiling for phenotype distinction

As for the study of different psoriasis types and severity phenotypes, an example is a study of 1990 which analyzed the concentration of essential fatty acids (EFAs) and their metabolites in plasma phospholipids by GC-MS in healthy individuals, and in patients with ichthyosis vulgaris, acne vulgaris or psoriasis [111]. In all three patient groups, concentrations of arachidonic and DPA were significantly lower than in the control group, suggesting that these abnormalities may occur in many skin diseases. However, concentrations of dihomogammalinolenic acid showed different pattern between the 3 skin diseases, suggesting that each may have different characteristic pattern of EFA metabolites. Moreover, psoriasis shows different severity phenotypes and can even cause systemic problem by affecting organs deeper than the skin. A more recent study focused on the metabolic profiling of psoriasis patients with different severity phenotypes performed an untargeted high-resolution LC-MS metabolomics analysis in order to measure plasma metabolites from 96 sex-matched individuals, 32 healthy donors, 32 mild and 32 severe psoriasis patients [105]. The results of this analysis indicated perturbations in 3 major amino acid metabolic pathways that were significantly associated with psoriasis, a) arginine and proline, b) glycine, serine and threonine, and c) alanine, aspartate, and glutamate. They also studied the effect of the anti-tumor necrosis factor (TNF)- α drug Etanercept treatment on the metabolic profiles of the patients. Etanercept treatment reversed the majority of psoriasis-associated metabolic phenotypes, shifting them from the ones of severe psoriasis toward that of healthy controls being significantly correlated with PASI clinical score. These results suggest that levels of circulating amino acids can be potentially used to monitor the severity of psoriasis, as well as serve as biomarkers for treatment response to anti-TNF α treatment. Although the responsible mechanism(s) are unclear, the altered amino acids levels in

psoriasis may be linked to the increased demand for collagen synthesis and keratinocyte hyperproliferation in psoriasis or the incidence of cachexia which is a common comorbidity of inflammatory conditions.

Organic acids in Psoriasis

Armstrong et al. aimed to discriminate between different psoriasis phenotypes and further elucidate the pathogenesis of psoriatic diseases by determining the differences in metabolomic profiles among psoriasis patients with or without psoriatic arthritis and healthy controls [106]. They performed metabolomic analysis by GC-TOF-MS of blood serum samples from 30 individuals age- and sex-matched divided in three groups, 10 patients with psoriasis, 10 patients with psoriasis and psoriatic arthritis and 10 healthy controls. Compared to the control group, psoriasis patients had a higher level of alpha ketoglutaric acid possible due to enhanced alpha ketoglutarate synthesis and lower level of asparagine and glutamine. As discussed previously, alpha-ketoglutaric acid increase could be explained by the increased cellular demand of amino acids, notably glutamine, due to high rates of cellular proliferation in psoriasis. Moreover, patients with psoriasis and psoriatic arthritis had increased levels of glucuronic acid compared to the control group, supporting a role of Glycosaminoglycans (GAGs) in psoriasis pathogenesis. Finally, patients with both psoriasis and psoriatic arthritis had decreased levels of alpha ketoglutaric acid and lignoceric acid, a very long-chain fatty acid, compared to patients with psoriasis alone. The authors suggested that alpha ketoglutarate can act to facilitate collagen synthesis in psoriasis patients and lower serum alpha ketoglutarate levels in the patients with both psoriasis and psoriatic arthritis may be the result of a higher inflammatory burden experienced by these patients. Moreover, Alonso et al. performed a large-scale profiling of the urine metabolome of six prevalent Immune-mediated inflammatory diseases (IMIDs): rheumatoid arthritis, psoriatic arthritis, psoriasis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis [112]. Initially, they analyzed the urine metabolome of 1210 patients and 100 controls from a 'discovery cohort', with two patient subgroups for each disease under investigation, one with high and one with low disease activity. For psoriasis, 101 patients with low disease activity and 84 with high disease activity participated. This analysis identified 28 urine metabolites significantly associated with disease diagnosis and 3 metabolites significantly associated with disease activity. Following this analysis, the most significant metabolite biomarkers were validated in an independent 'validation' cohort of 1200 patients and 200 controls, 26 for diagnostic associations and the 3 for associations with disease activity. For psoriasis the validation study included 100 with low and 92 with high disease activity. The combination of both analyses showed that several of the associated metabolites were part of metabolic pathways commonly altered in many IMIDs, including the citric acid cycle, phenylalanine, and glycine-serine metabolism

pathways. In particular, psoriasis was significantly associated with altered citrate, N-acetyl aminoacids, trigonelline, alanine, methylsuccinate and hippurate levels, a metabolic pattern also found in other IMIDs that were investigated, suggesting common biomarkers for early diagnosis and prevention of several IMIDs.

Unraveling the pathogenesis of Psoriasis using metabolomics

A limited number of studies have focused on the metabolomic profile of psoriatic comorbidities and revealed a heightened activity of inflammatory pathways and a possible link between gut microbiota and psoriasis development. The aim of Mysliwiec et al. was to study whether fatty acids are associated to psoriasis and its related comorbidities including obesity, type 2 diabetes and hypertension [113]. The researchers performed a targeted serum analysis of the concentrations of fatty acids in 85 patients with plaque psoriasis and 32 healthy controls and investigated their association with the disease severity, with markers of inflammation and possible involvement the aforementioned comorbidities. They analyzed the concentration and composition of 14 total serum fatty acids by GC- and LC-MS. All groups of fatty acids were analyzed, including saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), n-3 polyunsaturated FA (n-3 PUFA) and n-6 polyunsaturated FA (n-6 PUFA) and associated their profiles with certain psoriasis clinical characteristics, such as Psoriasis Area and Severity Index (PASI), Body Mass Index (BMI), inflammatory and biochemical markers, lipid profile and presence of psoriatic comorbidity. The levels of FAs were increased in all the patients with or without obesity compared to the control group. Moreover, the SFA/UFA ratio increased with the duration of the disease in all psoriatic patients. PASI score was associated with low levels of DHA and n-3 PUFA, and high levels of MUFA only in the non-obese patients. The authors suggested that FA metabolic pattern may play a role in the disease severity or incidence of comorbidities.

Finally, a very recent study investigated stress-associated disturbances in lipid metabolism /in mononuclear cells, mainly lymphocytes of patients with psoriasis vulgaris (Ps, n = 32) or with psoriatic arthritis (PsA, n = 16) compared to healthy individuals (n = 16) [114]. The results showed different phospholipid profiles in psoriatic patients reflecting disturbances in lipid metabolism. In particular, phosphatidylcholines and phosphatidylinositols containing linoleic, arachidonic, eicosatetraenoic and docosadienoic acids, were down-regulated in both groups of psoriatic patients compared to healthy individuals. In the case of non-enzymatic lipid metabolites associated with oxidative stress, 8-isoprostaglandin F₂ α (8-isoPGF₂ α) and free 4-hydroxynonenal (4-HNE) were higher in patients with psoriatic arthritis, while levels of 4-HNE-His were higher in patients with psoriasis vulgaris. In the case of the enzymatic lipid metabolism, increased levels of

endocannabinoids were observed in both forms of psoriasis, while higher expression of their receptors and activities of phospholipases activity were detected only in patients with psoriasis vulgaris. Moreover, cyclooxygenase-1 (COX-1) was enhanced in the psoriasis vulgaris patients, but cyclooxygenase-2 (COX-2) was enhanced both in patient groups, generating higher levels of eicosanoids. Finally, some major eicosanoids, such as 15-d-PGJ2 (15-deoxy- Δ 12,14-prostaglandin J2), 15-hydroxyeicosatetraenoic acid (15-HETE) were increased in psoriasis vulgaris patients and reduced in psoriatic arthritis patients. This study indicated discrete phospholipid metabolic profiles and differential stress responses to the 2 different forms of psoriasis with enhancement of immune system-modulating mediators in psoriatic mononuclear cells. These observations can be used for the evaluation of biomarkers that will be used in the diagnosis, prevention and therapeutic treatments of different psoriatic phenotypes.

Discussion

Overall, metabolomic studies performed in blood, urine and skin have revealed interesting metabolic perturbations in patients with psoriasis that can increase our understanding of the disease and indicate possible biomarkers for early diagnosis and treatment optimization. Arachidonic acid metabolism has been found to be affected in several studies both at skin and serum, even though the results are not always consistent. Arachidonic acid and linoleic acid are the major n-6 PUFAs which in turn are metabolized to pro-inflammatory eicosanoids under the activity of COXs, lipoxygenases (LOXs) and cytochrome P450s (CYPs). On the contrary, A-Linoleic and its derivatives, EPA and DHA are converted to the anti-inflammatory eicosanoids through enzyme competition. Because arachidonic acid production is regulated in an insulin-related manner the relative levels of AA can be a useful marker for insulin resistance and the related diseases [115]. Psoriasis patients are at increased risk of metabolic syndrome and diabetes, both of which have insulin resistance as a common denominator. One of the explanations is that the systemic body inflammation caused by the immune system response is an important underlying mechanism for the occurrence of insulin resistance, obesity and high blood pressure and possibly the link between psoriasis and the occurrence of metabolic syndrome. Studies have shown that the incidence of metabolic syndrome is higher in patients with psoriasis compared with people without psoriasis and the more severe the psoriasis, the higher are the chances for the patient to develop metabolic syndrome [116,117]. However, there is also evidence that psoriasis severity can be a predictor of insulin resistance independently of the presence of metabolic syndrome [118]. Additionally, beta cells in mice with psoriasis produced more insulin than those in the unaffected mice in a murine model of psoriasis, which could be due to the cells trying to compensate for insulin resistance observed in the psoriatic

mice [119]. Overall, there are several studies discussing the role of insulin resistance and obesity in psoriasis stressing the need to use advanced analytical tools, including metabolomics, to unravel their association.

In addition, it should be noted that amino acid metabolism is commonly affected in psoriasis and several theories have emerged. The observed high circulating levels of amino acids suggest that the nutrient-sensitive mTOR/S6K pathway may contribute to the risk of insulin resistance. The excess amino acid availability can stimulate the mTOR/S6K pathway and inhibit serine phosphorylation of insulin receptor substrate, which can lead to an impairment in insulin-stimulated glucose disposal in skeletal muscles and insulin-mediated inhibition of glucose production. In a study by Buerger et al, mTOR was found to be activated in the whole epidermis in psoriatic human skin, which has been suggested to lead to hyperplasia of epidermis and increased proliferation of undifferentiated keratinocytes [120].

As already discussed for asthma and COPD, altered plasma amino acid levels can be associated with cachexia that has not been widely studied alone from a metabolic perspective, and there is no common metabolic pattern on plasma levels of metabolites in cachectic patients or animal models [121]. Downregulation of circulating BCAAs concentrations observed in many chronic diseases, such as COPD, psoriasis and cancer, can be associated with cachexia that is a common comorbidity among them [72,73,77]. However, Kamleh et al. detected increase in circulating amino acids in psoriasis patients [105] which may not be related to cachexia, while O'Connell et al. reported changes in lipids, glycerol, and glucose, but not amino acids, in the plasma of a murine cancer cachexia model [122].

Despite these studies, additional work needs to be done to further validate the identified metabolic biomarkers, particularly in the setting of psoriasis treatment such anti-TNF- α therapy, as tested by Kamleh et al. In addition, the underlying mechanism by which these metabolites are involved in the pathogenesis of psoriasis needs to be determined. For this purpose, the development of a well-characterized metabolomics profile for patients with different psoriatic phenotypes and severity will contribute in understanding the pathophysiology of psoriasis and its associated comorbidities. In addition, large longitudinal studies with individuals with psoriasis before and after treatment may contribute to treatment optimization based on the personalized metabolic profile.

Hashimoto's Thyroiditis

Disease Background

Hashimoto's Thyroiditis (HT) is an autoimmune thyroid disease (AITD) and the most common cause of hypothyroidism. HT is one of the most common thyroid gland diseases and affects 3.5 cases per 1000 per year in women and 0.8 per 1000 per year in men Hashimoto Thyroiditis [123]. Another common AITD is Graves' disease (GD), which is the most common cause of hyperthyroidism [124,125]. Hyperthyroidism affects the 0.8–1.3% of an iodine sufficient population [124], while hypothyroidism is estimated to be around 1–1.5% [126]. Other thyroid disorders include subacute/de Quervain's thyroiditis, postpartum thyroiditis, and (multi-)nodular toxic goiter (TG) that are very prevalent diseases. The main cause of hypothyroidism is autoimmunity, while hyperthyroidism can be due to either an autoimmune process or a somatic de novo mutation of the follicular cells in case of TG [127].

In HT, thyroid cells are being assaulted through cell- and antibody-mediated mechanisms. The diagnosis of HT includes overt loss of thyrocytes observed in ultrasound, increased serum thyroid stimulating hormone TSH levels and circulating autoantibodies against Thyroglobulin (Tg) and thyroid peroxidase (TPO). HT has been associated with other endocrine diseases including Addison's disease, type 1 diabetes mellitus, and hypogonadism [128]. On the other hand, GD is characterized by thyroid growth through the binding of autoantibodies to TSH receptor and continuous thyroid hormone production. GD is the most common cause of thyrotoxicosis and is accompanied with thyroid eye disease (ophthalmopathy) and enlarged thyroid [129]. The thyroid gland plays an important role in the metabolism regulation and the manifestation of the disease usually occurs with a wide range of comorbidities that are associated with metabolism [130], while there is also evidence to be associated with psoriasis [131]. Importantly, in addition to the genetic predisposition, some prominent factors contributing to the onset of the disease are low metabolism (due to oxidation and deficiencies in vitamins and other elements) and insulin resistance (Vitamin D3 deficiency and psychogenic stress [132–134]. Long-term exposure to these factors affects the self-non-self discrimination ability of the immune system leading to the development of autoimmune thyroid disease. Importantly, hypothyroidism further aggravates the above factors by blocking metabolism, increasing both insulin resistance and resistance to vitamin D, also affecting the mood and cognition functions [129,134–136].

Due to the above, metabolomics can provide an explanation on the pathophysiology and metabolic changes in thyroid autoimmune diseases. In recent years, metabolomic analysis has been used to identify biomarkers for diagnosis and prevention of these diseases, but mainly to provide a comprehensive evaluation and comparison of the metabolic changes in patients with thyroid dysfunction induced by different autoimmune thyroid diseases [137–140].

Metabolomics in Hashimoto's Thyroiditis

The majority of recent metabolomics studies aim to identify differences between metabolic profiles of different autoimmune thyroid diseases, such as HT and GD, in order to contribute to early diagnosis of thyroid disorders and treatment response. A recent study aimed to provide a metabolomic analysis of patients with GD hyperthyroidism and patients with HT hypothyroidism [141]. This study performed serum metabolomics analysis by LC-MS, in 43 patients with GD hyperthyroidism, 45 patients with HT hypothyroidism, and 52 age- and sex-matched healthy controls. Among the 186 metabolites analyzed in all participants, amino acids, bile acids, free fatty acids, and lipids were included. The results of the analysis indicated that there were significant differences in 22 metabolites for the GD hyperthyroidism group and 17 metabolites for the HT hypothyroidism group, compared with the control group. Importantly, hyperthyroidism significantly affected arginine and proline metabolism and aminoacyl-transfer ribonucleic acid (tRNA) biosynthesis, while hypothyroidism had a significant impact on alanine, aspartate, and glutamate metabolism. On the other hand, consistent changes in PC (16:0/22:4), PC (18:2/20:4), and SM (d20:1/22:4) in the hyperthyroidism and hypothyroidism groups compared to the control group, suggested that thyroid hormone was not the only influencing factor and possibly thyroid autoimmunity was also involved. These results suggested that autoimmune thyroid dysfunction can alter the metabolic profile of the patients, while there are also discrete serum metabolic patterns among different thyroid autoimmune diseases. Moreover, Struja et al. assessed the ability of a high-throughput proton NMR metabolomic profile to distinguish disease type among GD ($n = 87$), HT ($n = 17$), toxic goiter ($n = 11$), subacute thyroiditis ($n = 4$) and postpartum thyroiditis ($n = 1$) [142]. This study aimed to assess diagnosis of thyroid disorder based on classical parameters, such as serum thyrotropin (TSH), free levothyroxine (fT4), antiTPO-Ab and TRAb levels. A sum of 227 metabolic biomarkers were quantified from serum using high-throughput proton NMR metabolomics, but failed to identify a metabolomic biomarker combination capable of predicting diagnosis.

Polyamines are indispensable polycations and some polyamine metabolites have been associated with autoimmune disorders. Song et al., aimed to identify the profile of polyamine metabolites in autoimmune thyroid disease (AITD) and their association with thyroid hormone, thyroid autoantibodies or disease progression [139]. A total of 136 individuals participated in the study, including 36 patients with GD, 33 patients with HT, 29 patients with thyroid autoantibody-positive (pTAb) and 38 age- and sex-matched healthy controls. Targeted metabolomics analysis was performed to measure 14 polyamine metabolites by LC-MS/MS, including polyamine precursors, polyamines and polyamine catabolite. Both GD and HT patients had increased levels of lysine, L-arginine, L-ornithine and agmatine and lower levels of N-acetylputrescine, putrescine, spermine and

1,3-diaminopropane compared with the control group. GD patients had significantly lower cadaverine level but higher spermidine, N-acetylspermidine and γ -aminobutyric acid level than the control group, while HT patients had significantly decreased N-acetylspermine compared to the controls. pTAB patients showed lower levels of spermine and N-acetylspermine compared to the controls. Moreover, the ratio of spermine: spermidine was significantly reduced in all the groups of patients, while spermine was negatively correlated with thyroid-specific antibodies grade, suggesting that thyroid autoimmunity is associated with low levels of spermine. Overall this study showed that most of the metabolites in GD and HT patients had similar profiles compared with the controls, suggesting that there could be a common pathophysiological basis or metabolic pathway involved in both diseases. However, as far as the metabolic profile of GD is concerned there are some inconsistencies between recent studies. For example, Song et al. showed that hyperthyroid patients had higher arginine and ornithine levels than healthy controls, while Chng et al. found that antithyroid drug treatment increased arginine levels during the transition from hyperthyroidism to euthyroidism in GD patients [140].

Finally, a separate study aimed to identify novel peripheral biomarkers of thyroid function, by identifying and evaluating metabolic patterns associated with Thyrotropin (TSH) and free thyroxine (FT4), important parameters for the evaluation of thyroid function. The researchers used an untargeted OMICS (proteomics and metabolomics) approach in a thyrotoxicosis model to identify novel biomarkers for thyroid function evaluation and early diagnosis [138]. They collected plasma from 16 healthy young men who were treated with levothyroxine (L-T4) for 8 weeks. Samples were collected at 3 timepoints, before the intake was started, during treatment and after its completion. Metabolites levels were correlated to FT4 serum concentrations in order to determine a molecular signature discriminating between thyrotoxicosis and euthyroidism. Treatment with L-T4 significantly altered the levels of 65 out of 349 detected metabolites, of which 45 had a positive and 20 a negative association with serum FT4. Most of the FT4-associated metabolites were lipids and related compounds, such as Free fatty acids (FFAs), acyl carnitines (ACs), PUFAs, lysophospholipids (LPs), and androgens. In general, the analysis identified several physiological metabolomic signatures indicating increased resting energy expenditure, induced defense against systemic oxidative stress, decreased lipoprotein particle levels, and increased levels of complement system proteins, as well as coagulation factors. Increased resting energy expenditure and enhanced mitochondrial fatty acid β -oxidation was due to increased long chain saturated and monounsaturated FFAs and glycerol levels, caused by levothyroxine treatment. Increased defense against systemic oxidative stress, was indicated by a strong positive FT4-association with γ -glutamyl amino acid (GGAA) levels. Importantly, a subset of 15 molecules allowed the creation of a strong prediction

model for thyroid hormone function independent of common TSH and FT4 measurements, which could be an important step towards the molecular characterization of early forms of hyperthyroidism. Further validation studies in larger cohorts of higher complexity in terms of age, sex and hypothyroid clinical conditions have to be performed. Another study aimed to examine the physiological, adipokine, and metabolomic changes occurring during GD transition from hyperthyroidism to euthyroidism with medical treatment [140]. Twenty-four Chinese women with an average age of 36.3 ± 8.6 years with newly diagnosed GD were recruited and treated with thioamides to achieve euthyroidism. Clinical parameters (body weight, resting energy expenditure etc), biochemical parameters (thyroid hormones, lipid profile, fasting insulin and glucose levels), serum leptin, adiponectin, and metabolomics profiles were measured during hyperthyroidism and early euthyroidism. The average duration of treatment required to reach euthyroidism was 38 ± 16.3 weeks. After treatment, a significant increase in body weight and fat mass, a reduction in resting energy expenditure and increase in respiratory quotient were observed. Moreover, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were significantly increased. Leptin levels were not altered, in contrast with adiponectin levels that were significantly increased. Similarly, fat-free mass, branched chain amino acid levels, or insulin sensitivity was not significantly affected, whereas fasting C2, medium-chain, long-chain, and total acylcarnitines were significantly reduced during treatment. The results of this study provide an insight on metabolomics and physiological changes in fuel metabolism and energy balance occurring during the transition from hyperthyroidism to euthyroidism and can be used for GD treatment selection and optimization.

Discussion

In conclusion, metabolomic studies on hyperthyroidism and hypothyroidism are very limited in number with a small contribution to clinical practice. Nevertheless, the results so far indicate that metabolic patterns are affected by thyroid dysfunction induced by autoimmune thyroid disease and some patterns are commonly observed in patients with thyroid dysfunction, or help to differentiate between different types of thyroid dysfunction. In particular, hyperthyroid patients show decreased levels of glycine and L-serine [143]. Since thyroid hormones are involved in the regulation of amino acid metabolism, it would be expected that hyperthyroidism and hypothyroidism show opposite amino acid metabolic patterns. However, Liu et al. showed that some amino acids appeared in a similar pattern in GD and HT patients, increased serum glutamine levels and decreased levels of L-glutamic acid, L-citrulline, and taurine. Glutamine levels may be involved in the link between thyroid autoimmunity and papillary carcinoma [144], as it acts as a metabolic fuel for cancer proliferation by activating mammalian target of rapamycin pathway [145]. In addition, Chng et al.

showed that there was no significant change in the levels of some serum amino acids in GD patients during the transition from hyperthyroidism to euthyroidism [140]. Altogether these data suggest that there may be a common pathophysiological pathway between GD and HT. However, these results require further investigation.

Fatty acid metabolism is also perturbed in thyroid dysfunction. Thyroid hormone promotes the uptake of free fatty acid in peripheral tissue, while it also stimulates lipolysis of white adipose tissue, which is the main source of circulating free fatty acids [146]. Therefore, hyperthyroidism should promote an increase in lipolysis of white adipose tissue followed by increased free fatty acid uptake in liver and muscle, whereas hypothyroidism leads to decreased lipolysis of white adipose tissue accompanied with a decreased uptake of free fatty acid in the liver [146,147]. However, it was shown that free fatty acids had similar profile in GD, HT and control group [141]. Moreover, thyroid hormone has also direct effects on lipid synthesis and metabolism and can lead to changes in phospholipid components by regulating multiple enzymes including desaturases, phospholipases, and acyltransferases [148,149]. It was recently shown that GD patients had increased lysophosphatidylcholine (LPC) and sphingomyelin (SM) levels after antithyroid treatment [150], while GD and HT patients had significantly different components of serum sphingolipids and phospholipids compared to the control group [141], suggesting that thyroid hormone together with thyroid autoimmunity can affect lipid metabolism.

In Hashimoto's thyroiditis as in many other autoimmune conditions, the immune system attacks the skin through inflammation chemicals promoting body inflammation. This is one of the explanations why patients with Hashimoto's thyroiditis, as patients with psoriasis, have a higher occurrence of cardiovascular disease, as well as obesity and metabolic syndrome. Psoriasis affects roughly 1-3% of the population and because it is an autoimmune condition, it is more common in patients suffering from Hashimoto's thyroiditis or Graves' disease. Notably, a study investigating thyroid abnormalities in psoriatic patients reported an association between the clinical characteristics of psoriasis and thyroid function [151]. It also reported increased thyroid hormone and anti-TPO levels in individuals with psoriasis, suggesting that thyroid hormones may trigger increased epidermal growth factor production, important for keratinocyte proliferation and relevant to the clinical manifestation of psoriasis. Moreover, Bianchi et al. have studied the association between thyroid disease and psoriatic arthritis in a retrospective study and found an increased TPO Ab levels in psoriatic arthritis patients compared to the control, while the average thyroid volume measured by ultrasound was higher among psoriatic arthritis patients [152].

As already discussed, patients with psoriasis are often affected by metabolic syndrome and obesity probably due to increased insulin resistance. Alidrisi et al. found a matched prevalence of obesity between psoriasis and control. Obese patients with psoriasis showed a significantly higher prevalence of TPO Ab, as compared to non-obese. Obesity and age at onset of (≥ 40 years) for psoriasis were associated with higher risk for development of TPO Ab. They also showed that obesity increases the risk of autoimmune thyroid diseases with an emerging role for leptin in thyroid autoimmunity [153]. Moreover, adipokines-derived cytokines, including leptin, are present in high concentrations in patients with psoriasis, possibly explaining the higher levels of TPO Ab in obese patients with psoriasis [154]. This association between psoriasis and Hashimoto's thyroiditis deserves clinical attention and may have impact on clinical research, prognosis and treatment selection. The presence of a more general but undiagnosed poly-autoimmunity could explain in many cases the prolonged symptoms, possible altered prognosis of the disease and poorer outcomes of treatment.

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Appendix 7 SPSS data base

Appendix 7 file containing SPSS databases (4) submitted separately.