Author/s:
Papamichael, MM;Katsardis, C;Lambert, K;Tsoukalas, D;Koutsilieris, M;Erbas, B;Itsiopoulos, C

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

Date:
2019-04-01

Citation:
Papamichael, M. M., Katsardis, C., 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. Journal of Human Nutrition and Dietetics, 32 (2), pp.185-197. https://doi.org/10.1111/jhn.12609.

Persistent Link:
https://hdl.handle.net/11343/284722

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

Maria Michelle Papamichael [1]; Charis Katsardis [3]; Katrina Lambert [2]; Dimitris Tsoukalas [4]; Michael Koutsilieris [3]; Bircan Erbas [2]; Catherine Itsiopoulos [1].

[1] La Trobe University, School of Allied Health, Department of Rehabilitation, 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

Keywords: Asthma; bronchial inflammation; children; fatty fish; omega-3 fatty acids

Acknowledgements: We gratefully acknowledge Basilopoulos and Sklavenitis supermarkets for their support to economically-disadvantaged families.

Source of funding: This research did not receive any specific grant from any funding agency commercial, or not-for-profit sectors. The first author is supported by an Australian Post Graduate Award PhD Scholarship administered by La Trobe University.

Declaration of interest: None.

Author Contributions:

MP is the principal author of the paper. CI is the principal investigator and DT, CK, MK, BE and MP co-investigators. BE and KL assisted with the statistical analysis and supported with the interpretation and revisions of the manuscript. All co-authors declare that we have seen and approved the final version of the manuscript being submitted. The authors confirm that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Corresponding Author:

Maria Michelle Papamichael

4 Ypereidou St, P.Faliro, 17563, Athens, Greece

Mob: 00306947073672

E-mail: sassipap@hotmail.com

MRS MARIA MICHELLE PAPAMICHAEL (Orcid ID : 0000-0003-1169-7141)

Article type : Original Research

ABSTRACT

Background

Childhood asthma is the most common respiratory disorder worldwide associated with increased morbidity and decreased quality of life. Omega-3 fatty acids have anti-inflammatory and immunomodulating properties, however, their efficacy in asthma is controversial. The objective of this study was to examine the efficacy of a Mediterranean diet supplemented with a high omega-3 'fatty' fish intake in Greek asthmatic children.

Methods

A single-centred, six-month parallel randomized controlled trial comparing consumption of a Mediterranean diet supplemented with two meals of 150g cooked fatty fish weekly (intervention) with usual diet (control) on pulmonary function in children (5-12 years) with mild asthma. Pulmonary function was assessed using spirometry and bronchial inflammation by Fractional exhaled Nitric Oxide (FeNO) analysis.

Results

Sixty-four children, (52% male, 48% female) successfully completed the trial. Fatty fish intake increased in the intervention group from 17 grams/day at baseline to 46 grams/day at six months ($p<0.001$). In the unadjusted analysis the effect of the intervention was of borderline significance ($p= 0.06$, $\beta= -11.93$; 95%CI: -24.32, 0.46). However, after adjusting for age, sex, BMI and regular physical activity a significant effect was observed ($p=0.04$, $\beta= -14.15$ ppb; 95% CI: -27.39, -0.91). No difference was observed for spirometry, asthma control and quality of life scores.

Conclusions

A Mediterranean diet supplemented with two fatty fish meals per week might be a potential strategy to reduce airway inflammation in childhood asthma. Future robust clinical trials are warranted to replicate and corroborate these findings.

Keywords: Asthma; bronchial inflammation; children; fatty fish; Omega-3 fatty acids

INTRODUCTION

Childhood asthma has become the most common respiratory disorder worldwide [1] as well as in Greece [2] associated with increased morbidity and poor quality of life. Asthma causes substantial physical, mental and economic burden due to increased rates of hospitalization, emergency visits for medical care, school absence and parent's time off work [3]. According to the Global Initiative for Asthma (GINA), it has been estimated that by 2025 there will an additional 100 million people suffering with asthma [4]. Therefore, identifying potential asthma therapies is of great public health significance.

Asthma is a heterogeneous disease caused by genetic and environmental factors. A multitude of environmental factors have been associated with asthma risk, namely respiratory infections, smoking, pollution, pet hair, house dust mite, mould and diet [5]. There is evidence that diet can influence the development and progression of asthma in children. In general, a diet that is high in fat, processed foods, sugar and salt has been shown to increase the prevalence and risk of asthma in children and adolescents [6; 7; 8; 9; 10]. In contrast, the International Study of Allergies and Asthma in Childhood (ISAAC) showed that regular intake of fruit, vegetables, fish and adherence to the Mediterranean diet have a prophylactic effect on asthma in children and adolescents [6; 11; 12; 13; 14]. In particular, lower prevalence of asthma was found in Mediterranean centres of Western Europe that share a common dietary pattern [15]. The term 'Mediterranean diet' refers to dietary patterns found in olive-growing areas of the Mediterranean region. The key features of the traditional Mediterranean diet are: a high intake of vegetables, wild edible greens, fruits, unrefined cereals, bread, legumes and olives that are fresh, seasonal, locally grown and minimally-processed as well as an abundance of olive oil.; low to moderate intake of dairy, poultry, fish (depending on the proximity of the sea), nuts, and seeds; low intake of meat and sweets including regular intake of wine with meals [16]. This diet is rich in monounsaturated fatty acids, a balanced ratio of n-6: n-3 essential fatty acids and high amounts of fibre and antioxidants such as vitamins E and C, resveratrol, polyphenols, selenium, glutathione that interact synergistically promoting good health [16].

Research studies have confirmed that excessive amounts of omega-6 fatty acids leading to a high omega-6: omega-3 fatty acid ratio, can promote the pathogenesis of chronic diseases including asthma [17]. 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(common in Western diets) had adverse consequences [18]. Thus, indicating that an optimal ratio of these two fatty acids, and high intake of omega-3 fatty acids might have a prophylactic potential on asthma symptoms. Fatty fish (salmon, mackerel, herring, sardines and trout) is a rich source of long-chain omega-3 fatty acids, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). One fish meal can provide between 1.5-3.0 g of EPA/DHA and one gram fish oil capsule per day approximately 300 mg [19]. Most of the epidemiological evidence which gave rise to the hypothesis that marine omega-3 fatty acids might have a prophylactic effect, was generated from observational studies reporting that early introduction in life and regular consumption of fish in children had a protective effect on asthma in children up to 14 years old [20]. Thus, suggesting that fish which consists of an array of bioactive nutrients including EPA, DHA and antioxidants might have a different health impact as compared to fish oil supplementation [21; 22]. However, to date there are no universal dietary guidelines for asthma and the efficacy of marine omega-3 fatty acid therapy in asthma has not been well established. Further research is required to validate this therapy. Exploring the potential of non-pharmacological treatments is important due to the comparatively low risk associated with their use. A dietary modification could reduce asthma burden and improve the quality of life in children suffering with asthma. The objective of this study was to investigate the efficacy of a Mediterranean diet supplemented with high omega-3 'fatty' fish intake in Greek asthmatic children.

METHODS

Study design

The present study was a six-month parallel Randomized Controlled Trial (RCT) investigating the effect of the Greek Mediterranean diet supplemented with high omega-3 'fatty' fish intake on asthma in children. The study design has been described in detail elsewhere using CONSORT recommendations [23] and only key features are presented here [24]. This RCT was conducted according to ethical standards in the Declaration of Helsinki and all procedures involving human subjects were approved by the institutional review board of La Trobe University Human Ethics Committee. The study protocol was registered with the Australian and New Zealand Clinical Trial Registry (www.ANZCTR.org.au/**ACTRN12616000492459p)**.

Participants

Seventy-two children (54.60% boys, 46.40 % girls, mean age 7.98 ± 2.24 years old) with asthma were recruited from a paediatric asthma clinic in the greater city of Athens, Greece from 1st November to 31st December, 2016. An internet platform (http://www.randomization.com) was used to automate the random assignment of patient number to randomization number which was linked to the intervention arms. Eligible participants were randomized equally to intervention or control groups with a 1:1 allocation ratio by the physician, after written informed consent was obtained from parents. Inclusion criteria included children aged between 5-12 years and having physician–diagnosed "mild" asthma as defined by the Global Initiative for Asthma (GINA) guidelines [(1)]. According to GINA, 'mild asthma' is 'well-controlled' asthma. A patient that has day symptoms and need for reliever medication less than twice a week, no night-waking symptoms or limitations in daily activities due to asthma is considered to have 'mild asthma'[(1)]. The exclusion criteria were children with severe or chronic asthma [(25)], gastroesophageal reflux disease, cystic fibrosis, congenital respiratory disease [(1)], food allergies, taking multiple glucocorticoid medication, high-dose multi-vitamins or fish oil supplements as well as being vegetarian and not willing to modify their diet.

Intervention

The intervention group was instructed to consume two fatty fish meals per week (at least 150g cooked fish) [(26)] as part of the Greek Mediterranean diet over a period of 6 months [(27)]. In comparison, the control group consumed their usual diet. The intervention group was provided with detailed dietary education delivered by a dietician on the key principles of a Mediterranean diet including a pamphlet indicating the types of fatty fish to be consumed (such as sardines, trout, salmon, mackerel, anchovies), amounts of fresh fish equivalent to 150 g cooked fillet fish and a list of lean fish not to be consumed over the study period. In order to monitor and facilitate intervention compliance, parents were issued a table to record the type and amount of fatty fish consumed per meal as well as the two days per week that fatty fish was consumed during the six month period. This record was returned to the dietician on a monthly basis and collected at the end of the six month study. In comparison, the control group was instructed to consume their usual diet and was provided with advice on general healthy dietary guidelines according to the Hellenic Ministry of Health and Welfare (1999) [(27)].

Measurements

Children were assessed at two time-points, baseline and at six months during usual medical consultations. Since children were younger than 12 years of age, parents were used as a proxy to complete questionnaires[24]. During the same week of medical consultations, a telephone interview was conducted by the dietician to collect information regarding the participant's medical history, medicine use and adherence to the Mediterranean diet. Dietary intake was measured using a Food Frequency Questionnaire that is based on the validated semi-quantitative PANACEA-FFQ for Greek children 10-12 years old [28] and adherence to the Mediterranean diet using the KIDMED tool [29]. Throughout the study, all participants were monitored fortnightly by the dietician via telephone, e-mails, text and face-to-face consultation. Any missing data was retrieved during telephone interviews.

Food intake calculations

Daily consumption of each food item as grams per day was assessed from FFQs. The reported frequency of consumption of FFQ items was converted to frequency of consumption per day.

Then grams per day were calculated by multiplying portion size by the value corresponding to each consumption frequency [28]. Based on the participant's responses the information was then aggregated into food groups. Eleven main food groups (Dairy products, fruit, vegetables, legumes, starch, meat, sweets, fast food, savoury snacks, fats and soft drinks) were formed reflecting a dietary pattern followed by the population.

Anthropometry

Children's height was measured to the nearest 0.1 cm using a SECA stadiometer after shoes had been removed and children were positioned in the standard Frankfort horizontal plane [30]. Body weight was measured to the nearest 0.1 kg on calibrated electronic scales (SECA, Hanover, MD) without shoes and heavy clothing. Body mass index (BMI) was calculated (kg/m^2) and study participants were classified normal weight, overweight and obese using the Hellenic paediatric growth charts [31].

Pulmonary function and bronchial inflammation

Pulmonary function was measured by trained technicians according to European Respiratory Society (ERS) protocol [32] using a portable spirometer (MIR Spirobank II, MIR Inc., USA) providing age, gender, weight and height. 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 of three technically acceptable tests was selected. Normal pulmonary function was considered values of FEV_1 greater than 80% predicted and variation in FEV_1 of 10-12% to be clinically significant in children [33].

Levels of FeNO were measured by FeNO analyser (NO Breath, Benfont Inc., UK) following ATS/ERS guidelines [34]. Absence of lung inflammation and good asthma control was indicated by FeNO values less than 20 ppb [35; 36].

Questionnaires

Asthma Control

Asthma Control was evaluated using the Greek translation of the Asthma Control Questionnaire (ACQ) which is a validated questionnaire assessing asthma control in paediatric patients aged 6-12 years old[37]. A score < 0.75 is considered as having 'well-controlled' asthma and a score of ≥ 1.5 indicates 'extremely poorly controlled' [37].

Quality of Life

Children's quality of life was measured using the Greek translation of the validated mini Paediatric Asthma Quality of Life Questionnaire (PAQLQ) for asthmatic children aged 6-16 years [38; 39]. Parents assisted children in completing questionnaires. Children were asked to recall their experiences during the past week and to respond to each question on a scale from 1-7, where 1 indicates severe impairment and 7 no impairment [39].

Adherence to Mediterranean dietary pattern

Adherence to the Mediterranean dietary pattern was measured using the KIDMED Index which is a 16-item test that has been developed specifically for Spanish children and adolescents [29] and has been applied previously to assess Mediterranean diet compliance in Greek children and adolescents [40; 41]. The KIDMED score ranges from 0-12. A score of 0-3

reflects low Mediterranean diet adherence,4-7 improvement needed and 8-12 optimal Mediterranean diet [29].

Physical activity status

Physical activity status was estimated using the International Study of Asthma and Allergies in Childhood (ISAAC) Phase 3 Environmental Questionnaire [42]. Regular physical activity was considered to be more than or equal to three times per week.

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 [43] (Supplement 1).

Sample size

Sample size was based on spirometry measure, FEV_1 and was determined using G Power Analysis [44]. Assuming a modest effect size of 0.4 [45] to show a significant difference in FEV_1, we estimated that a sample of at least 64 patients was adequate to provide a power of 90%, to evaluate two-sided hypotheses regarding statistically differences in FEV_1 between groups at a probability level less than 0.05 and allowed for a 20% drop out rate.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS version 20.0, IBM Corp, Armonk NY) software. Continuous variables were assessed for normality using Shapiro-Wilks test and are presented as means and standard deviation. And categorical

variables are shown as frequencies. Differences between the intervention groups were compared using t-test for normally-distributed variables and Mann-Whitney test or Chi-square test otherwise. The effect of the intervention on pulmonary function, asthma control and quality of life was assessed using multiple linear regression models controlling for potential confounding factors including age, sex, physical activity and BMI. The results from the regression model are presented as unstandardized β coefficients and corresponding 95% CI. The level of statistical significance was defined at $P< 0.05$. According to the American Thoracic Society (ATS) guidelines, a reduction in FeNO by at least 10 units for values lower than 50 ppb as the cut point to indicate a significant response to anti-inflammatory therapy [36].

RESULTS

At baseline, 72 children were recruited and randomly allocated into two groups (Figure1). Sixty-four children (51.6% male and 48.4% female) of which thirty-one children were in the intervention group and thirty-three in the control, completed the trial and baseline and follow-up assessments. The overall participation rate was 88.9% (64/72). Eight children dropped out, one due to allergy (not related to the intervention) and seven for personal reasons.

FIGURE 1 HERE

At baseline, homogeneity between the groups was observed for demographic and clinical characteristics as well as for adherence to the Mediterranean dietary pattern (Table 1).

TABLE 1 HERE

The same trend was observed in the six month follow-up, except for the KIDMED score. There was a modest improvement in adherence to the Mediterranean dietary pattern from baseline to follow-up for the intervention group as compared to the control [KIDMED score: 5.32 to 6.10 (intervention group) vs 5.24 to 5.12 (control group); p=0.02] most likely due to increased fish intake, although scores indicate improvement in Mediterranean diet adherence is needed.

Dietary intake of main food groups, fatty/lean fish, nuts, olive oil, fats, sweets, fast food, savoury snacks and soft drinks are presented in Table 2 whereas adherence to the Mediterranean diet in Table 3.

.

TABLE 2 HERE

Table 2 shows a statistically significant increase in fatty fish intake for the intervention group ($p<0.001$) from approximately 17 grams /day at baseline to 46 grams/day at six months as compared to the control group 10 grams/day at baseline and six months. Hence, validating good compliance to the dietary intervention by the intervention group. The same trend is apparent from the KIDMED questionnaire in Table 3. A statistically significant increase ($p<0.001$) in frequency of consumption of fish (at least 2-3 times per week) from approximately 13% at baseline to 84% at six months was observed in the intervention group as compared to 6.1% at both time-points in the control group.

TABLE 3 HERE

Regarding biochemical tests, at baseline, no significant difference in plasma fatty acid composition was observed between the groups ($p>0.05$).In comparison, at six months significant differences in DHA ($p<0.001$), total plasma omega-3 fatty acids levels ($p<0.001$) and omega-6: omega-3 fatty acid ratio ($p<0.001$) were observed between the groups (Table 4). The percentage change in DHA was 119.80% in the intervention group and 43.39% in the control (crude analysis).As for EPA levels, differences between the groups were not significant. At six months, total plasma omega-3 fatty acid levels were higher in the intervention group as compared to the control (mean value: 168.20 vs 115.82 mcmol/L respectively); and the omega- 6: omega-3 fatty acid ratio was lower in the intervention group than in the control [(14.5:1) vs (19.4:1)] respectively. Elevated levels of total plasma omega-3 fatty acids (or lower omega-6 to omega-3 fatty acid ratio) in the intervention group is a marker of compliance to the dietary intervention, thus confirming that biomarkers are a good surrogate for dietary intake [46]

TABLE 4 HERE

As for asthma control and quality of life mean change in scores from baseline to follow-up were not significantly different between the two groups in the univariate and multivariate analysis ($p>0.05$)(Table 5). The same trend was noted for spirometry. No significant change in spirometry from baseline was observed between groups in the univariate and multivariate analysis (Table 5).

TABLE 5 HERE

Regarding bronchial inflammation, crude analysis showed that FeNO increased by 78.23% in the control group, but decreased by 18.56% for the intervention group (Supplement 2).

When applying Multiple Linear Regression model, in the unadjusted analysis the effect of the intervention was of borderline significance (p= 0.06, β= -11.93; 95%CI: -24.32, 0.46). However, after adjusting for age, sex, BMI and regular physical activity a significant effect was observed (p=0.04, β= -14.15 ppb; 95% CI: -27.39, -0.91). Specifically, two meals of fatty fish (at least 150g cooked filleted fish/meal) in the context of a Mediterranean diet resulted in a decrease in bronchial inflammation as measured by FeNO by 14.15 ppb (95% CI: -27.39, -0.91; β= -14.15; p=0.04) (Table 6).

TABLE 6 HERE

Medication use

Regarding asthma medication use, at baseline, 53 out of 64 children were taking medication as part of their daily asthma therapy, of which 51 children were taking anti-leukotriene agonists [intervention (24) vs control (27)]. At six months 35 out of 64 children were taking medication [intervention (14) vs control (21)], with 31 children taking anti-leukotriene therapy (intervention (12) vs control (19). In the crude analysis, a greater reduction in anti-leukotriene use from baseline to six months was seen in the intervention group as compared to the control (24 vs 12; 27 vs 19 respectively), although the result was not significant (X^2 test: p=0.21).

DISCUSSION

The present clinical trial explored the hypothesis that a Mediterranean diet supplemented with high omega-3 fatty acid intake as 'fatty' fish improves pulmonary function and decreases symptoms in asthmatic children. The KIDMED score revealed that in both the intervention and control groups and across all time-points children did not have optimal adherence to the Traditional Mediterranean diet. Previous studies undertaken in Mediterranean regions have reported an abandonment of the Mediterranean dietary pattern in children and adolescents [47]. Nevertheless, the most significant finding of our study is that fatty fish intake, specifically eating two meals of fatty fish (at least 150g cooked fillet fish per meal) weekly in the context of a Mediterranean diet, resulted in a decrease in bronchial inflammation (FeNO) by 14 units in the intervention group. According to the American Thoracic Society (ATS) guidelines, a reduction in FeNO by at least 10 units for values lower than 50 ppb as the cut point indicate a significant response to anti-inflammatory therapy [36]. No statistical significant differences in spirometry, asthma control or quality of life were observed between the groups. A possible explanation why we did not observe any change in spirometry with fatty fish intake could be because children had normal lung function and well-controlled asthma. On the other hand, our recent meta-analysis investigating the role of fish intake in childhood asthma documented that early introduction and regular intake of fish (at least once a week) was beneficial on 'current wheeze' (OR, 0.62; 95% CI, 0.48-0.80) and 'current asthma' (OR, 0.75; 95% CI, 0.60-0.95) in children up to 4.5 years old, whereas fatty

fish intake was protective for ‘ current asthma’ in children 8-14 years old (OR, 0.35; 95% CI, 0.18-0.67) [20]. However, in the majority of these studies asthma outcome was assessed using a questionnaire and not by spirometry.

Another finding worth noting that warrants further exploration was that there was a higher reduction in medication use for children in the intervention group as compared to the control. Dotterud et al, (2013) reported that fatty fish intake reduced asthma medication use in the last 12 months among girls at age 2 years [48]. Hence, proposing that medication use could be decreased in some patients with asthma with increased dietary omega-3 fatty acid intake from fatty fish if both the drug and omega-3 fatty acids exert their therapeutic effects though the same molecular actions. Thus, the possibility exists for a synergistic effect of drug-diet interactions that confer greater anti-inflammatory benefits when combined than either intervention alone or similar effects with less side-effects.

Regarding the slight increase in plasma DHA concentration (43%) observed in the control group, a plausible explanation for this increase is that DHA was derived from other foods. It has been reported that DHA status can be improved by long-term intake of vegetable oils. However, the increase in tissue DHA may not be immediate and not as effective as direct consumption of DHA from fish or fish oil supplements [17]. The Mediterranean dietary pattern is a varied diet that consists of high intake of vegetables, wild edible greens; low to moderate intake of free-range animal products such as meat, poultry, and eggs [16] that are terrestrial sources of DHA, although not as much as in fatty fish. For example Atlantic cod contains 277mg DHA per 180 g fillet, 29 mg in 180g chicken breast, 12 mg in an egg, 2 mg in a pork chop and 1 mg in 90g beef steak as compared to 2477 mg in 180g of farmed Atlantic salmon [49]. In contrast for the intervention group, increased fatty fish intake a rich source of DHA/EPA resulted in approximately 120% increase in plasma DHA along with a decrease in bronchial inflammation biomarker FeNO.

Several mechanisms have been proposed by which marine omega-3 fatty acids decrease bronchial inflammation in asthma. In vitro experiments have demonstrated that consumption of fish leads to a shift in omega-3/omega-6 fatty acid balance resulting in reduced production of inflammatory mediators involved in disease development [17]. Fatty fish is rich in EPA and DHA, which can inhibit cyclooxygenase and lipo-oxygenase enzyme activity, and decrease pro-inflammatory mediators from n-6 fatty acid arachidonic acid such as 2-series

prostaglandins(PGE2) and 4-series leukotrienes (leukotriene E4, leukotriene B4), eosinophils and TNF-α that promote airway oedema, mucus secretion, bronchial inflammation, bronchospasm and onset of asthma symptoms [19].The 2-series PG have an immune-modulatory function, which modifies the activity of macrophages and lymphocytes and suppresses the production of T-helper 1 (Th1)-related cytokines, promoting the expression of the T-helper 2 (Th2) phenotype [19]. Th2 responses include the production of interleukin IL-4, IL-5, IL-9, and IL-13, and are associated with increased levels of Ig E by B lymphocytes and eosinophil production leading to severe bronchial inflammation and onset of asthma symptoms[50; 51]. Furthermore, EPA gives rise to eicosanoids with lower biological potency than those generated from arachidonic acid [19], thus are weaker inducers of inflammation. Unique to EPA and DHA, both these fatty acids are precursors to resolvins, and DHA to protectins and maresins that are mediators with anti-inflammatory resolving properties .For example, DHA derived molecule, resolvin D1, facilitates the phagocytic engulfment and clearance of apoptotic neutrophils which is essential in the resolution of inflammation [52], along with reduced eosinophil activation and infiltration into the lung, decreased IL-5 concentration, Th2 cytokines, Th17, airway mucus metaplasia as well as airway hyperactivity and promoted inactivity of pro-inflammatory transcription activator Nuclear Factor kappa B (NFkB) . In addition, DHA has an important role in oxidative stress associated with inflammation [53; 54]. DHA exerts anti-oxidant effects by reducing the intracellular accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as maintaining optimal levels of glutathione and anti-oxidant enzymes [52]. During inflammation, excessive production of nitric oxide (NO) in the lungs causes tissue damage. DHA is able to inhibit the expression of inducible nitric oxide synthase (iNOS), an enzyme responsible for NO production [52].

Strengths/limitations

To our knowledge this is the first clinical trial investigating the effect of high omega-3 ‘fatty’ fish intake added to a Mediterranean diet in children with “mild” asthma. Few studies have examined the effect of fatty fish in asthma [26; 48; 55; 56] and our study adds to the existing evidence. A strength in our study is that we used fish as opposed to fish oil in the dietary intervention. It has been reported that fish consumption can significantly increase serum levels of DHA and EPA in humans compared to fish oil supplementation [52]. Fish is a source of high quality protein and trace minerals especially selenium and iodine which are not provided in fish oil supplements that may have other beneficial effects. In contrast, fish oil

584 supplements might not provide sufficient anti-inflammatory activity because of impaired
585 enzymatic activity in asthma patients [57]. Also fish oil is known to have unpleasant taste,
586 odour and adverse effects such as gastrointestinal disturbances [58; 59] and therefore is less
587 palatable and not sustainable. Another strength of this study is that pulmonary function and
588 bronchial inflammation were assessed quantitatively as compared to parent's report of
589 symptoms or use of a questionnaire. Treatment and monitoring of asthma are guided by
590 symptom scores or lung function parameters which are not always accurate markers of
591 disease severity [35]. Exhaled nitric oxide is an important biomarker for bronchial
592 inflammation in asthma since a patient can be asymptomatic and spirometry may not always
593 reflect the underlying inflammation [35]. Also, it is valuable in identifying eosinophilic
594 inflammation, adherence and effectiveness of medication therapy and in predicting risk of
595 future exacerbations [60]. In this study patient adherence was assessed by independent dietary
596 biomarkers rather than using dietary data [46]. Furthermore, plasma fatty acid composition is a
597 reliable indicator of dietary fat intake in children [61].

598

599 The primary weakness of this study is the short duration period and possible limited power
600 to adjust for multiple confounders and conduct analysis of effect modification. Changes in
601 inflammatory cytokines were not measured which could have added to the extent of
602 inflammation reduction. It would have been interesting to examine whether fish oil in fatty
603 fish could suppress the production of cytokines to levels similar to those attained with
604 appropriate asthma therapy and is associated with clinical improvement. Another drawback is
605 that questionnaires were self-administered by parents which might have led to
606 misinformation and recall bias. In addition, at baseline, FeNO was higher in the intervention
607 group and lower in the control group and it is possible that the difference in FeNO was driven
608 by the increase in the control group and regression to the mean. Nevertheless, at baseline,
609 there was no bronchial inflammation in both groups since FeNO was less than 20 ppb.
610 Moreover, we may have not taken all potential confounding variables into consideration such
611 as environmental tobacco exposure, maternal education, residence area, parental atopic
612 disease, social economic status and number of siblings. However, the multivariate analysis
613 decreases the probability of confounding and an effort was made to correct for age, sex,
614 regular physical activity and BMI. A potential issue for some families may have been the
615 time required for preparation and cooking of fish meals. Nevertheless, the health benefits
616 would outweigh the burden.

In conclusion, our findings suggest that a Mediterranean diet supplemented with fatty fish might be a potential non-pharmacological strategy to combat airway inflammation. This has important public health implications because dietary interventions are easily applied in 'real-life' situations, are of low cost, have multiple health benefits, and might assist in reducing asthma burden in children. Given that there are no adverse effects of regular fish consumption, a healthy diet incorporating two fatty fish meals per week provides overall health benefits and well-being. Future robust clinical trials are warranted to replicate and corroborate the promising findings documented.

Transparency statement

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with CONSORT guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

REFERENCES

1. GINA (2016). Global Strategy for Asthma Management and Prevention, 2016. www.ginasthma.org (accessed June 2018).

2. Anthracopoulos MB, Fouzas S, Pandiora A et al. (2011). Prevalence trends of rhinoconjunctivitis, eczema, and atopic asthma in Greek schoolchildren: four surveys during 1991-2008. Allergy Asthma Proc **32**, 56-62.

3. Nunes C, Pereira AM, Morais-Almeida M (2017). Asthma costs and social impact. Asthma Res and Prac **3**, 1.

4. Masoli M, Fabian D, Holt S et al. (2004). Global Burden of Asthma. http://www.ginasthma.com (accessed June 2018).

5. Beasley R, Semprini A, Mitchell EA (2015). Risk factors for asthma: is prevention possible? Lancet **386**, 1075-1085.

6. Ellwood P, Asher MI, García-Marcos L et al. (2013). Do fast foods cause asthma, rhinoconjunctivitis and eczema? Global findings from the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. Thorax **68**, 351-60.

7. Patel S, Custovic A, Smith JA et al. (2014) Cross-sectional association of dietary patterns with asthma and atopic sensitization in childhood - in a cohort study. Pediatr Allergy Immunol **25**, 565-571.

8. Berentzen NE, van Stokkom VL, Gehring U et al. (2015). Associations of sugar-containing beverages with asthma prevalence in 11-year-old children: the PIAMA birth cohort. Eur J Clin Nutr **69**, 303-308.

9. Arvaniti F, Priftis KN, Papadimitriou A et al. (2011). Salty-snack eating, television or video-game viewing, and asthma symptoms among 10-to 12-year-old children: the PANACEA study. J Am Diet Assoc **111**, 251-257.

10. Rodriguez-Rodriguez E, Perea JM, Jimenez AI et al. (2010). Fat intake and asthma in Spanish schoolchildren. Eur J Clin Nutr **64**, 1065-1071.

11. Papamichael MM, Itsiopoulos C, Susanto NH et al. (2017). Does adherence to the Mediterranean dietary pattern reduce asthma symptoms in children? A systematic review of observational studies. Public Health Nutr **20**, 2722-2734.

12. Chatzi L, Torrent M, Romieu I et al. (2007). Diet, wheeze, and atopy in school children in Menorca, Spain. Pediatr Allergy Immunol **18**, 480-485.

13. Saadeh D, Salameh P, Caillaud D et al. (2015). Prevalence and association of asthma and allergic sensitization with dietary factors in schoolchildren: data from the french six cities study. BMC Public Health **15**, 993.

14. Nagel G, Weinmayr G, Kleiner A et al. (2010). Effect of diet on asthma and allergic sensitisation in the International Study on Allergies and Asthma in Childhood (ISAAC) Phase Two. Thorax **65**, 516-522.

15. ISSAC (1998). Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J **12**, 315-335.

16. Willett WC, Sacks F, Trichopoulou A et al. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr **61**, 1402s-1406s.

17. Simopoulos AP (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) **233**, 674-688.

18. Broughton KS, Johnson CS, Pace BK et al. (1997). Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr **65**, 1011-1017.

19. Calder PC (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta **1851**, 469-484.

20. Papamichael MM, Shrestha SK, Itsiopoulos C et al. (2018). The role of fish intake on asthma in children: A meta-analysis of observational studies. Pediatr Allergy Immunol **29**, 350-360.

21. Muley P, Shah M, Muley A (2015). Omega-3 Fatty Acids Supplementation in Children to Prevent Asthma: Is It Worthy?-A Systematic Review and Meta-Analysis. J Allergy (Cairo) **2015**, 312052.

22. Thien FCK, De Luca S, RK W et al. (2011). Cochrane Review: Dietary marine fatty acids (fish oil) for asthma in adults and children. Evid-Based Child Health **6**, 984-1012.

23. Schulz KF, Altman DG, Moher D (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ **340,** c332.

691 24. Papamichael MM, Katsardis C, Tsoukalas D et al. (2018). A clinical trial of
692 Mediterranean diet enriched with fatty fish in pediatric asthma: Study protocol. J Pharm
693 Pharmacol **6**, 225-239.

694 25. Chung KF, Wenzel SE, Brozek JL et al. (2014). International ERS/ATS guidelines on
695 definition, evaluation and treatment of severe asthma. Eur Respir J **43**, 343-373.

696 26. Hodge L, Salome C, Peat J et al. (1996). Consumption of oily fish and childhood asthma
697 risk. Medical J Aust **164**, 137-140.

698 27. Hellenic Ministry of Health & Welfare (1999). Dietary guidelines for adults in Greece.
699 Archives of Hellenic Medicine **16**, 516-524.

700 28. Antonogeorgos G, Grigoropoulou D, Papadimitriou A et al. (2013). Validation of a food
701 frequency questionnaire designed for children 10-12 years: THE PANACEA-FFQ
702 PERIOPERATIVE NURSING **2,** 40-54.

703 29. Serra-Majem L, Ribas L, Ngo J et al. (2004). Food, youth and the Mediterranean diet in
704 Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and
705 adolescents. Public Health Nutr **7**, 931-935.

706 30. WHO (2008). Training Course on Child Growth Assessment. .
707 http://www.who.int/childgrowth/training/en/ (accessed June 2018).

708 31. Hellenic Institute of Child Health (ICH) (2017). Hellenic pediatric growth charts in Child
709 Health Book. www.ygeiapaidiou-ich.gr (accessed June 2018).

710 32. Miller MR, Hankinson J, Brusasco V et al. (2005). Standardisation of spirometry. Eur
711 Respir J **26**, 319-338.

712 33. Katsardis C, Alexandraki S, Paraskakis E (2015). Chapter 2: Spirometry in children 6-16
713 years old. In: Katsardis Ch, Koumbourlis A, Anthracopoulos M, Paraskakis. Paediatric
714 Pulmonary Function Testing Indications and Interpretation, pp 15-42. New York, USA:
715 NOVA Biomedical.

716 34. ATS/ERS (2005). ATS/ERS recommendations for standardized procedures for the online
717 and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide,
718 2005. Am J Respir Crit Care Med **171**, 912-930.

719

720 35. Paraskakis E, Katsardis C, Chatzimichail A (2015). Chapter 17: Exhaled NO. In:
721 Katsardis Ch, Koumbourlis A, Anthracopoulos M, Paraskakis. Paediatric Pulmonary
722 Function Testing Indications and Interpretation, pp 235-248. New York, USA: NOVA
723 Biomedical.

724 36. Dweik RA, Boggs PB, Erzurum SC et al. (2011). An official ATS clinical practice
725 guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J
726 Respir Crit Care Med **184**, 602-615.

727 37. Juniper EF, Gruffydd-Jones K, Ward S et al. (2010). Asthma Control Questionnaire in
728 children: validation, measurement properties, interpretation. Eur Respir J **36**, 1410-1416.

729 38. Juniper EF, Guyatt GH, Feeny DH et al. (1996). Measuring quality of life in children
730 with asthma. Qual Life Res **5**, 35-46.

731 39. Wing A, Upton J, Svensson K et al. (2012). The standardized and mini versions of the
732 PAQLQ are valid, reliable, and responsive measurement tools. J Clin Epidemiol **65**, 643-650.

733 40. Antonogeorgos, Panagiotakos D, Grigoropoulou D et al. (2014). Investigating the
734 Associations between Mediterranean Diet, Physical Activity and Living Environment with
735 Childhood Asthma using Path Analysis. Endocr Metab Immune Disord Drug Targets **14**,
736 226-233.

737 41. Arvaniti F, Priftis KN, Papadimitriou A et al. (2011). Adherence to the Mediterranean
738 type of diet is associated with lower prevalence of asthma symptoms, among 10-12 years old
739 children: the PANACEA study. Pediatr Allergy Immunol **22**, 283-289.

740 42. International Study of Asthma and Allergies in Childhood (ISAAC) (2012). ISAAC
741 Phase 3 Environmental Questionnaire.
742 http://isaac.auckland.ac.nz/phases/phasethree/environmentalquestionnaire/environmentalques
743 tionnaire.html (accessed June 2018).

744 43. Stellaard F, ten Brink HJ, Kok RM et al. (1990). Stable isotope dilution analysis of very
745 long chain fatty acids in plasma, urine and amniotic fluid by electron capture negative ion
746 mass fragmentography. Clin Chimica Acta **192**, 133-144.

747 44. Faul F, Erdfelder E, Lang AG et al. (2007). G*Power 3: a flexible statistical power
748 analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods **39**,
749 175-191.

45. Lee SC, Yang YH, Chuang SY et al. (2013). Reduced medication use and improved pulmonary function with supplements containing vegetable and fruit concentrate, fish oil and probiotics in asthmatic school children: a randomised controlled trial. Brit J Nutr **110**, 145-155.

46. Potischman N (2003). Biologic and methodologic issues for nutritional biomarkers. J Nutr **133**, 875s-880s.

47. Grosso G, Galvano F (2016). Mediterranean diet adherence in children and adolescents in southern European countries. NFS Journal **3**, 13-19.

48. Dotterud CK, Storro O, Simpson MR et al. (2013). The impact of pre- and postnatal exposures on allergy related diseases in childhood: a controlled multicentre intervention study in primary health care. BMC Public Health **13**, 123.

49. USDA (2018). Foods High in DHA (Docosahexaenoic Acid). USDA National Nutrient Database for Standard Reference, Release 28. https://ndb.nal.usda.gov/ndb/nutrients/index (accessed July 2018)

50. Berger A (2000). Th1 and Th2 responses: what are they? BMJ **321**, 424.

51. Finn PW, Bigby TD (2009). Innate immunity and asthma. Proc Am Thorac Soc **6**, 260-265.

52. Yum HW, Na HK, Surh YJ (2016). Anti-inflammatory effects of docosahexaenoic acid: Implications for its cancer chemopreventive potential. Semin Cancer Biol. **40-41**, 141-159.

53. Rogerio AP, Haworth O, Croze R et al. (2012). Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. J Immunol **189**, 1983-1991.

54. Sun YP, Oh SF, Uddin J et al. (2007). Resolvin D1 and its aspirin-triggered 17R epimer. Stereochemical assignments, anti-inflammatory properties, and enzymatic inactivation. J Biol Chem **282**, 9323-9334.

55. Oien T, Storrø O, Johnsen R (2010). Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study. J Epidemiol Community Health **64**, 124-129.

56. Kiefte-de Jong JC, de Vries JH, Franco OH et al. (2012). Fish consumption in infancy and asthma-like symptoms at preschool age. Pediatrics **130**, 1060-1068.

779 57. Miyata J, Arita M (2015). Role of omega-3 fatty acids and their metabolites in asthma
780 and allergic diseases. Allergol Int **64**, 27-34.

781 58. Abedi E, Sahari MA (2014). Long-chain polyunsaturated fatty acid sources and
782 evaluation of their nutritional and functional properties. Food Sci Nutr **2**, 443-463.

783 59. Sidhu KS (2003). Health benefits and potential risks related to consumption of fish or fish
784 oil. Regul Toxicol Pharmacol **38**, 336-344.

785 60. Petsky HL, Kew KM, Chang AB (2016). Exhaled nitric oxide levels to guide treatment
786 for children with asthma. Cochrane Database Syst Rev **11**, CD011439.

787 61. Moilanen T, Rasanen L, Viikari J et al. (1992). Correlation of serum fatty acid
788 composition with dietary intake data in children and young adults. Ann Med **24**, 67-70.

Table 1. Demographics, clinical tests and Mediterranean diet adherence at baseline and six months

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS PER INTERVENTION GROUP										
	BASELINE					SIX MONTHS				
	Group					Group				
	Intervention		Control			Intervention		Control		
Variable	Mean	SD	Mean	SD	P[a]	Mean	SD	Mean	SD	P[a]
Age (years)	7.78	2.25	8.18	2.26	0.47	8.19	2.18	8.76	2.35	0.30[b]
Male n (%)	12 (38.7%)		21 (63.6%)		0.05					
Height (cm)	133.70	13.48	133.30	13.62	0.91	136.40	13.50	135.8	13.67	0.98[b]
BMI (kg/m^2)	18.62	3.95	18.20	3.81	0.72	18.70	3.90	18.73	3.84	0.98[b]
Overweight/obese*	15(48.4%)		11 (33.4%)		0.22	13(41.9%)		14 (42.4%)		0.86
Regular physical activity (%)										
(≥ 3x/wk)	13 (41.9%)		19 (57.6%)		0.46	13 (41.9%)		18 (54.5%)		0.59
Medication n (%)						14 (45.1%)		21(63.6%)		0.18
Yes	26(83.9%)		27(81.8%)		0.83					
Anti-leukotriene therapy n (%)	24 (77%)		27(81.8%)		0.16	12 (38.7%)		19(57.5%)		0.21
Pulmonary function										
FEV_1 (% predicted)	97.23	8.80	99.09	10.55	0.45	100.19	9.44	100.09	8.76	0.96
FVC (% predicted)	94.61	8.68	96.30	11.11	0.50	96.94	9.20	96.79	9.14	0.95
FEV_1/FVC (% predicted)	101.97	4.40	102.39	7.78	0.79	102.87	3.66	102.88	5.60	0.99
PEF (% predicted)	94.32	19.28	93.48	18.79	0.86	100.58	21.02	101.21	21.68	0.91
FEF_{25-75} (% predicted)	100.29	17.80	103.94	21.47	0.46	103.45	14.09	102.73	19.78	0.87

FeNO (ppb)	17.94	17.61	10.15	7.16	0.16[b]	14.61	15.07	18.09	29.41	0.81[b]
ACQ score	0.35	0.34	0.36	0.39	0.96	0.23	0.49	0.20	0.28	0.87[b]
PAQLQ score	6.77	0.32	6.70	0.44	0.47	6.83	0.56	6.90	0.18	0.91[b]
KIDMED Score	5.32	2.01	5.24	2.02	0.87	6.10	1.49	5.12	1.98	**0.02[b]**

Bold characters represent statistical significant p-values.

Key: ACQ- Asthma Control Questionnaire; PAQLQ- Paediatric Asthma Quality of Life Questionnaire; FEV_1- Forced Expiratory Volume in 1 second; FVC- Forced Vital Capacity; FEV_1/FVC- ratio of Forced Expiratory Volume and Forced Vital Capacity; PEF- Peak Expiratory Flow; $FEF_{25-75\%}$-Mid Expiratory Flow 25-75% vital capacity; FeNO- Fractional exhaled Nitric Oxide analysis. P[a] - P-values shown were calculated using t-test or Chi-square test; P[b] -P-value estimated using non-parametric Mann-Whitney test. *Hellenic paediatric growth charts [(38)]

Table 2.Consumption of main food groups and items (grams per day) baseline and six months

	CONSUMPTION OF FOOD GROUPS AND FISH									
	BASELINE					SIX MONTHS				
	Group					Group				
	Intervention		Control			Intervention		Control		
Food group/Item (grams/day)	Mean	S.D	Mean	S.D	P[b]	Mean	SD	Mean	SD	P[b]
Dairy products	486.91	301.96	513.21	305.61	0.71	453.09	283.90	517.24	303.22	0.38
Fruit	282.33	139.18	251.94	172.36	0.23	270.25	187.53	259.08	187.70	0.65
Vegetables	167.74	129.24	174.68	133.47	0.85	172.58	103.97	206.23	155.48	0.49
Legumes	65.49	35.32	56.07	33.70	0.22	59.81	32.22	46.81	31.75	**0.03**
Starch	113.47	131.38	67.02	25.56	0.03	94.18	59.38	75.78	24.63	0.42
Meat	84.03	47.03	81.04	38.43	0.77	69.60	30.80	82.88	45.93	0.27
Seafood	7.02	11.14	4.65	6.89	0.45	5.60	6.98	7.09	6.57	0.29
Lean fish	17.54	11.73	11.71	13.63	0.01	15.77	25.69	13.20	10.50	0.09
Fatty fish	17.18	11.79	10.41	11.62	**0.01**	45.76	19.40	10.35	11.01	**0.00**
Nuts	3.01	6.45	5.12	6.46	0.09	3.81	6.90	4.45	5.58	0.38
Olive oil	16.43	12.49	19.34	14.76	0.31	18.08	14.96	18.98	14.02	0.98
Fats	20.28	14.38	25.87	19.48	0.32	24.01	19.43	24.88	15.92	0.49
Fast food	19.73	16.99	26.81	36.49	0.64	19.61	12.84	23.75	20.36	0.65
Sweets	25.82	17.50	27.89	17.71	0.63	20.58	14.98	29.89	29.03	0.22

Savoury snacks	27.22	31.78	43.97	41.89	0.03	28.93	24.55	39.26	35.64	0.16
Soft drinks	8.52	16.02	30.08	47.50	0.07	36.14	72.29	36.02	46.38	0.28

P[b]- P-value calculated with Mann-Whitney test

Table 3. KIDMED Questionnaire baseline and six month follow-up

KIDMED QUESTIONNAIRE							
		BASELINE			SIX MONTHS		
		Group			Group		
Question	Response	Intervention % (n)	Control % (n)	P[a]	Intervention % (n)	Control % (n)	P[a]
Q1. Does your child take a fruit or fruit juice every day?	Yes	80.6% (25)	81.8% (27)	0.90	87.1% (27)	84.8% (28)	0.79
Q2. Eat two fruits every day?	Yes	61.3% (19)	60.6 % (20)	0.95	48.4 % (15)	57.6% (19)	0.46
Q3. Eat fresh salad or cooked vegetables regularly once a day?	Yes	58.1% (18)	54.5% (18)	0.78	54.8% (17)	45.5% (15)	0.45
Q4. Eat fresh salad or cooked vegetables more than once a day	Yes	6.5% (2)	9.1% (3)	0.69	6.5% (2)	12.1% (4)	0.44
Q5. Eat fish regularly (at least 2-3 times per week)?	Yes	12.9% (4)	6.1% (2)	0.35	83.9% (26)	6.1% (2)	**0.00**
Q6. Go to a fast-food restaurant (hamburger) more than once a week?	Yes	3.2% (1)	6.1% (2)	0.59	0	9.1% (3)	0.09
Q7. Eats legumes more than once a week	Yes	32.3% (10)	36.4% (12)	0.73	19.4%(6)	15.2% (5)	0.66
Q8. Eats pasta or rice almost every day (5 or more times per week)?	Yes	12.9% (4)	6.1% (2)	0.35	25.8% (8)	6.1% (2)	**0.03**
Q9. Eats cereals or grains (bread etc.) for breakfast?	Yes	35.5% (11)	48.5% (16)	0.29	35.5% (11)	45.5% (15)	0.42
Q10. Eat dairy products for breakfast?	Yes	80.6% (25)	87.9% (29)	0.43	80.6% (25)	90.9% (30)	0.24
Q11. Eat baked goods or pastries for breakfast?	Yes	3.2% (1)	3.0% (1)	0.96	0.0% (0)	3% (1)	0.33
Q12. Skips breakfast?	Yes	12.9% (4)	12.1% (4)	0.92	12.9% (4)	9.1% (3)	0.62

Q13. Eat nuts regularly (at least 2-3 times per week)?	Yes	6.5% (2)	15.2% (5)	0.26	12.9% (4)	12.1% (4)	0.92
Q14. Eat 2 yogurts and/or some cheese (40g) daily?	Yes	83.9% (26)	78.8% (26)	0.60	80.6% (25)	78.8% (26)	0.85
Q15. Eat sweets and candy several times every day?	Yes	19.4% (6)	39.4% (13)	0.08	12.9% (4)	21.2% (7)	0.38
Q16. Eat olive oil with meals?	Yes	100% (31)	100% (33)	k	100% (31)	100% (33)	k

P[a] - P-value calculated using Chi Square Test; k- constant

Table 4. Plasma fatty acid composition of children at baseline and six months per group

	PLASMA FATTY ACIDS COMPOSITION									
	BASELINE					SIX MONTHS				
	Group					Group				
	Intervention		Control			Intervention		Control		
Plasma fatty acid (mcmol/L)	Mean	S. D	Mean	S.D	P[b]	Mean	S.D	Mean	S.D	P[b]
Omega-3 fatty acids										
alpha-Linolenic acid	10.81	5.0	12.33	4.94	0.38	14.72	5.23	14.40	4.20	0.91
EPA	30.22	13.53	33.01	21.57	0.54	29.99	15.78	25.08	8.10	0.33
DHA	54.54	30.94	50.07	33.67	0.33	119.88	41.43	71.80	26.36	**0.00**
Omega-6 fatty acids										
gamma- Linolenic acid	10.15	6.92	11.67	6.35	0.25	25.80	16.01	28.37	19.86	0.79
Linoleic acid	1,124.29	443.25	1.147.32	428.68	0.87	1,724.34	546.64	1,602.04	433.88	0.34
Arachidonic acid	446.55	643.37	362.12	85.64	0.24	381.54	84.42	411.59	122.76	0.33
Total plasma fatty acids	4,939.01	1,084.76	5,118.12	1,175.32	0.64	5,973.66	1,391.99	5,678.64	1,229.67	0.62

Total plasma Ω3 fatty acids	99.17	46.61	99.45	57.31	0.35	168.20	56.40	115.82	34.61	**0.00**
Total plasma Ω6 fatty acids	1,557.90	443.45	1,618.86	479.28	0.79	2,254.36	607.29	2,167.93	547.56	0.62
Ratio Ω6 : Ω3	24.24	26.48	23.21	25.81	0.13	14.48	4.92	19.44	4.64*	**0.00**

P[b] - P-values estimated using Mann-Whitney test; PUFA-polyunsaturated fatty acids; Ω3- omega-3 fatty acids; Ω6- omega-6 fatty acids

Table 5.Univariate and Multivariate analysis showing mean change in asthma control, quality of life and pulmonary function parameter estimates from baseline to follow-up in the intervention and control groups.

Mean change	Group		Difference			
	Intervention	Control	Mean	95% CI	P^a	P^c
ACQ score	-0.13	-0.16	0.03	-0.19; 0.24	0.80	0.66
PAQLQ score	0.05	0.19	-0.14	-0.34; 0.05	0.15	0.23
FVC (% predicted)	2.45	-0.12	2.57	-1.81; 6.96	0.24	0.33
FEV_1 (% predicted)	2.84	0.61	2.23	-1.87; 6.34	0.28	0.38
FEV_1/FVC (%predicted)	0.58	0.79	-0.21	-2.54; 2.12	0.86	0.86
PEF % (%predicted)	6.06	7.06	-0.99	-11.18; 9.18	0.85	0.71
FEF_{25-75} (% predicted)	2.35	-1.24	3.60	-3.09; 10.28	0.29	0.44
FeNO (ppb)	-3.84	8.09	-11.93	-24.32; 0.46	0.06	**0.04**

Key: ACQ- Asthma Control Questionnaire score; PAQLQ- Paediatric Asthma Quality of Life Questionnaire score. FEV_1- Forced Expiratory Volume in 1 second; FVC- Forced Vital Capacity; FEV_1/FVC- ratio of Forced Expiratory Volume and Forced Vital Capacity; PEF- Peak Expiratory Flow; $FEF_{25-75\%}$- Mid Expiratory Flow 25-75% vital capacity; FeNO- Fractional exhaled Nitric Oxide analysis.

P^a- P-value calculated using t-test;

P^c- P-value from multiple linear regression analysis adjusted for confounders age, sex, BMI and regular physical activity.

Table 6. Multiple linear regression analysis showing mean change in bronchial inflammation (FeNO) for intervention and control groups from baseline to follow-up

Mean score change	Variable	Difference		
		β	95% CI	P^c
FeNO(ppb)				
	Group	-14.15	-27.39; -0.91	**0.04**
	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

Key: β-unstandardized beta; FeNO- Fractional exhaled Nitric Oxide analysis

P^c - P-value evaluated applying multiple linear regression model adjusted for confounders age, sex, BMI and regular physical activity.

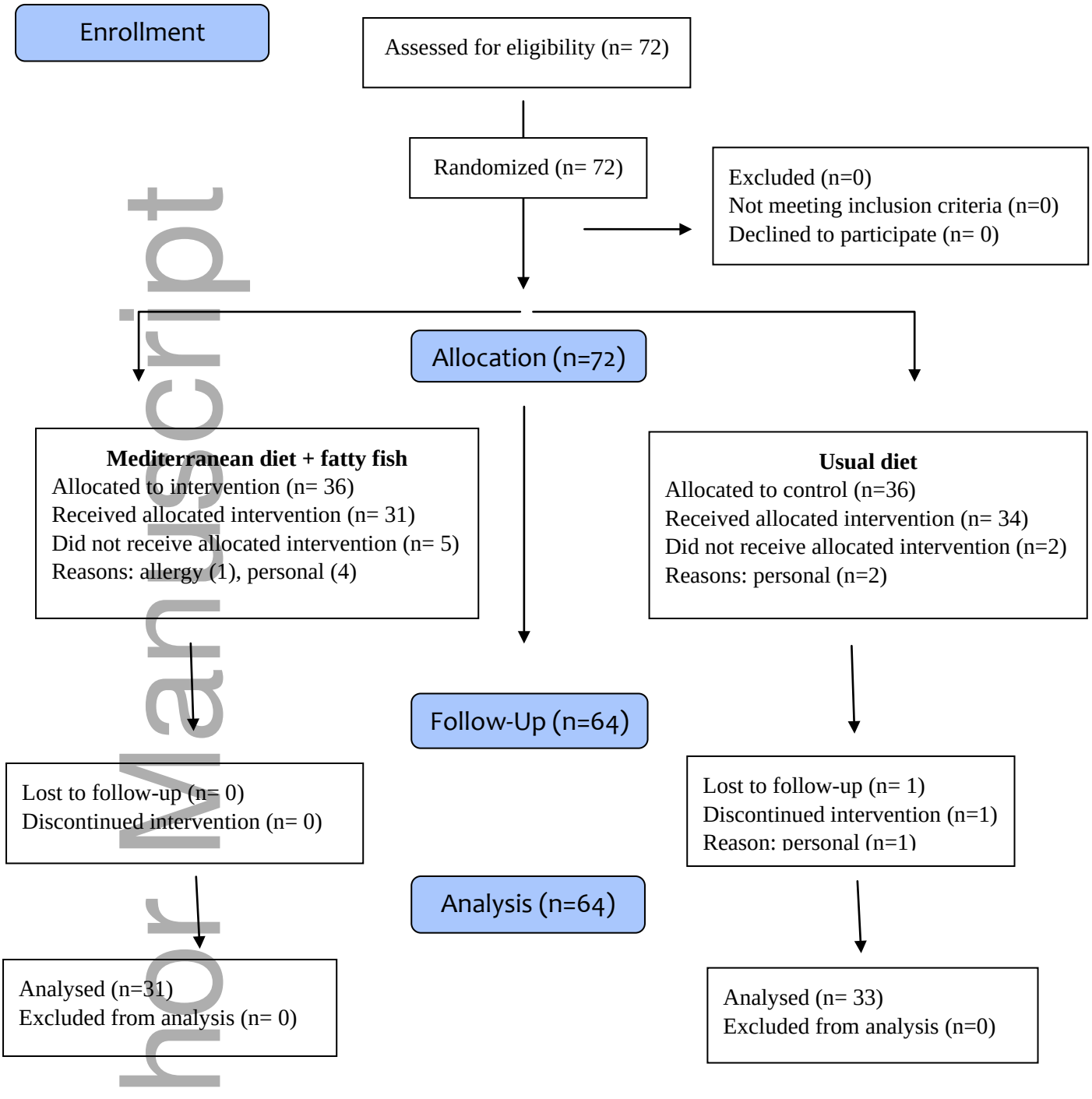

Figure 1. Consort flow diagram of study design [23]