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RESEARCH PAPER

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

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Abstract

Background: Childhood asthma is the most common respiratory disorder worldwide, being associated with increased morbidity and a decreased quality of life. Omega-3 fatty acids have anti-inflammatory and immunomodulating properties; however, their efficacy in asthma is controversial. The present study aimed 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, 6-month, parallel randomised controlled trial compared the consumption of a Mediterranean diet supplemented with two meals of 150 g of cooked fatty fish weekly (intervention) with the usual diet (control) with respect to pulmonary function in children (aged 5–12 years) with mild asthma. Pulmonary function was assessed using spirometry and bronchial inflammation by fractional exhaled nitric oxide analysis.
Results: Sixty-four children (52% male, 48% female) successfully completed the trial. Fatty fish intake increased in the intervention group from 17 g day^{-1} at baseline to 46 g day^{-1} at 6 months ($P < 0.001$). In the unadjusted analysis, the effect of the intervention was of borderline significance ($P = 0.06$, $\beta = -11.93$; 95% confidence interval = −24.32 to 0.46). However, after adjusting for age, sex, body mass index and regular physical activity, a significant effect was observed ($P = 0.04$, $\beta = -14.15$ ppb; 95% confidence interval = −27.39 to −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 for reducing airway inflammation in childhood asthma. Future robust clinical trials are warranted to replicate and corroborate these findings.

Introduction

Childhood asthma has become the most common respiratory disorder worldwide [(1)], as well as in Greece [(2)], being associated with increased morbidity and a poor quality of life. Asthma causes substantial physical, mental and economic burden as a result of increased rates of hospitalisation, 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 mites, 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 [6–10]. By contrast, the International Study of Allergies and Asthma in Childhood (ISAAC) showed that a regular intake of fruit, vegetables and fish, as well as adherence to the Mediterranean diet, has a prophylactic effect on asthma in children and adolescents [6,11–14]. In particular, a 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; a low to moderate intake of dairy, poultry, fish (depending on the proximity of the sea), nuts and seeds; and a low intake of meat and sweets, including a 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, which interact synergistically to promote 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 (as in common in Western diets) had adverse consequences [18]. Thus, an optimal ratio of these two fatty acids and a high intake of omega-3 fatty acids might have prophylactic potential with respect to 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 1 g of fish oil capsule per day can provide approximately 300 mg [19]. Most of the epidemiological evidence giving rise to the hypothesis that marine omega-3 fatty acids might have a prophylactic effect was generated from observational studies reporting that an early introduction in life and regular consumption of fish in children had a protective effect on asthma in children aged up to 14 years old [20]. Thus, fish, consisting of an array of bioactive nutrients, including EPA, DHA and antioxidants, might have a different health impact 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 nonpharmacological treatments is important because of 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 present study aimed to investigate the efficacy of a Mediterranean diet supplemented with high omega-3 'fatty' fish intake in Greek asthmatic children.

Materials and methods

Study design

The present study was a 6-month parallel randomised 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 in accordance with the 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 (SD) age 7.98 (2.24) years] with asthma were recruited from a paediatric asthma clinic in the greater city of Athens, Greece from 1 November to 31 December, 2016. An internet platform (http://www.randomization.com) was used to automate the random assignment of a patient number to a randomisation number, which was linked to the intervention arms. Eligible participants were randomised 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 GINA guidelines [1]. According to GINA, 'mild asthma' is 'well-controlled' asthma. A patient that has day-time symptoms and the need for relief medication

less than twice a week, as well as no night-waking symptoms or limitations in daily activities as a result of asthma, is considered to have 'mild asthma' [1]. 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 multivitamins 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 150 g of 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 a detailed dietary education delivered by a dietitian 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 and anchovies), amounts of fresh fish equivalent to 150 g of cooked fillet fish and a list of lean fish not to be consumed over the study period. 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 2 days per week that fatty fish was consumed during the 6-month period. This record was returned to the dietitian on a monthly basis and collected at the end of the 6-month study. In comparison, the control group was instructed to consume their usual diet and was provided with advice on general healthy dietary guidelines in accordance with the Hellenic Ministry of Health and Welfare (1999) [27].

Measurements

Children were assessed at two time-points: baseline and at 6 months during usual medical consultations. Because 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 dietitian 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 (FFQ) based on the validated semi-quantitative PANACEA-FFQ for Greek children aged 10–12 years [28] and adherence to the Mediterranean diet using the KIDMED tool [29]. Throughout the study, all participants were monitored fortnightly by the dietitian via telephone, e-mails, text and face-to-face consultation. Any missing data were retrieved during telephone interviews.

Food intake calculations

Daily consumption of each food item (g day^{-1}) was assessed from FFQs. The reported frequency of consumption of FFQ items was converted to frequency of consumption per day.

Then g day^{-1} was 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 stadiometer (Seca Corp., Hanover, MD, USA) after their 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 Corp.) 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., New Orleans, LA, USA) providing age, sex, 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 forced expiratory volume in 1 s (FEV_1) greater than 80% predicted and variation in FEV_1 of 10–12% to be clinically significant in children [33].

Levels of fractional exhaled nitric oxide (FeNO) were measured using a FeNO analyser (NO Breath, Benfont Inc., UK) in accordance with American Thoracic Society (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 [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 to 7, where 1 indicates severe impairment and 7 indicates 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 to 12. A score of 0–3 reflects low Mediterranean diet adherence, 4–7 indicates improvement is needed and 8–12 reflects an optimal Mediterranean diet [29].

Physical activity status

Physical activity status was estimated using the 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 2 h after the last meal before testing. Venous samples (4 mL) were collected from children following a 2-h 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 the case of haemolysis, blood collection was repeated. The internal standard mixture (200 μL of methyl nonadecanoate in hexane containing butylated hydroxytoluene) was added to 100 mL of plasma. Fatty acid hydrolysis and derivatisation 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 fatty acid methyl esters was performed using hexane. These were then transferred to gas chromotography 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] (see Supporting information, Appendix S1).

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% dropout rate.

Statistical analysis

Data were analysed using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA) software. Continuous variables were assessed for normality using the Shapiro–Wilks test and are presented as the mean (SD). And categorical variables are shown as frequencies. Differences between the intervention groups were compared using *t*-test for normally-distributed variables and a Mann–Whitney test or chi-squared 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 unstandardised β coefficients and corresponding 95% confidence interval (CI). $P < 0.05$ was considered statistically significant. According to the ATS guidelines, a reduction in FeNO by at least 10 units for values lower than 50 ppb should be used as the cut-off point to indicate a significant response to anti-inflammatory therapy [36].

Results

At baseline, 72 children were recruited and randomly allocated into two groups (Fig. 1). Sixty-four children (51.6% male and 48.4% female), of whom 31 children were in the intervention group and 33 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 as a result of an allergy (not related to the intervention) and seven for personal reasons.

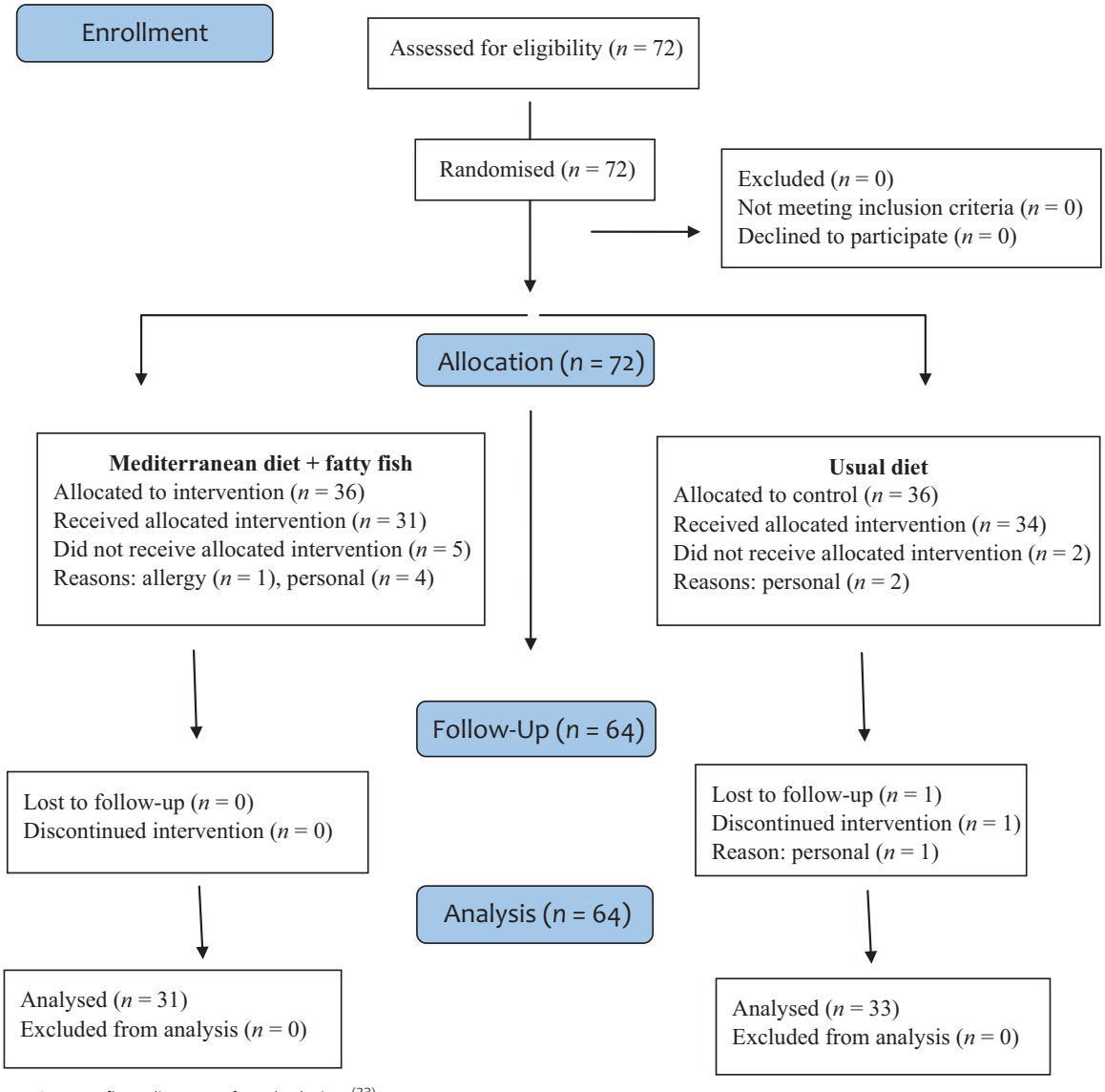

Figure 1 Consort flow diagram of study design [23].

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).

The same trend was observed in the 6-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 compared to the control group [KIDMED score: 5.32–6.10 (intervention group) versus 5.24–5.12 (control group); $P = 0.02$], most likely as a result of increased fish intake, although scores indicate that an improvement in Mediterranean diet adherence is needed.

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

Table 2 shows a statistically significant increase in fatty fish intake for the intervention group ($P < 0.001$) from approximately 17 g day^{-1} at baseline to 46 g day^{-1} at 6 months compared to the control group (10 g day^{-1} at baseline and 6 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 the frequency of consumption of fish (at least two or three times per week) from approximately 13% at baseline to 84% at 6 months was observed in the intervention group compared to 6.1% at both time-points in the control group.

Table 1 Demographics, clinical tests and Mediterranean diet adherence at baseline and 6 months

	Demographic and clinical characteristics per intervention group									
	Baseline group					Six months group				
	Intervention		Control			Intervention		Control		
Variable	Mean	(SD)	Mean	(SD)	*P**	Mean	(SD)	Mean	(SD)	*P**
Age (years)	7.78	(2.25)	8.18	(2.26)	0.47	8.19	(2.18)	8.76	(2.35)	0.30†
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†
BMI (kg m^{-2})	18.62	(3.95)	18.20	(3.81)	0.72	18.70	(3.90)	18.73	(3.84)	0.98†
Overweight/obese‡	15(48.4%)		11 (33.4%)		0.22	13(41.9%)		14 (42.4%)		0.86
Regular physical activity (%) (≥3 × week)	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†	14.61	(15.07)	18.09	(29.41)	0.81†
ACQ score	0.35	(0.34)	0.36	(0.39)	0.96	0.23	(0.49)	0.20	(0.28)	0.87†
PAQLQ score	6.77	(0.32)	6.70	(0.44)	0.47	6.83	(0.56)	6.90	(0.18)	0.91†
KIDMED score	5.32	(2.01)	5.24	(2.02)	0.87	6.10	(1.49)	5.12	(1.98)	**0.02**†

Bold characters represent statistical significant *P*-values.

ACQ, asthma control questionnaire; BMI, body mass index; $FEF_{25–75\%}$, mid expiratory flow 25–75% vital capacity; FeNO, fractional exhaled nitric oxide analysis; FEV_1, forced expiratory volume in 1 s; FEV_1/FVC, ratio of forced expiratory volume and forced vital capacity; FVC, forced vital capacity; PAQLQ, paediatric asthma quality of life questionnaire; PEF, peak expiratory flow.

**P*-values shown were calculated using *t*-test or chi-squared test.

†*P*-value estimated using nonparametric Mann–Whitney test.

‡Hellenic paediatric growth charts [38].

Regarding biochemical tests, at baseline, no significant difference in plasma fatty acid composition was observed between the groups ($P > 0.05$). In comparison, at 6 months, significant differences in DHA ($P < 0.001$), total plasma omega-3 fatty acids levels ($P < 0.001$) and the 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). Regarding EPA levels, differences between the groups were not significant. At 6 months, total plasma omega-3 fatty acid levels were higher in the intervention group compared to the control group (mean value: 168.20 versus 115.82 μmol L^{-1}, respectively) and the omega- 6: omega-3 fatty acid ratio was lower in the intervention group than in the control (14.5 : 1 versus 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 are a marker of compliance to the dietary intervention, thus confirming that biomarkers are a good surrogate for dietary intake [46]

Regarding asthma control and quality of life, the mean change in scores from baseline to follow-up was 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).

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 (see Supporting information, Fig. S1).

When applying the multiple linear regression model, in the unadjusted analysis, the effect of the intervention was of borderline significance ($P = 0.06$, $\beta = -11.93$; 95% CI = −24.32 to 0.46). However, after adjusting for age, sex, BMI and regular physical activity, a significant effect

Table 2 Consumption of main food groups and items (grams per day) baseline and 6 months

	Consumption of food groups and fish									
	Baseline group					Six-month Group				
	Intervention		Control			Intervention		Control		
Food group/Item (g day^{-1})	Mean	(SD)	Mean	(SD)	*P**	Mean	(SD)	Mean	(SD)	*P**
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

Bold characters represent statistical significant *P*-values.
**P*-value calculated with Mann–Whitney test.

was observed ($P = 0.04$, $\beta = -14.15$ ppb; 95% CI = −27.39 to −0.91). Specifically, two meals of fatty fish (at least 150 g of 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 to −0.91; $\beta = -14.15$; $P = 0.04$) (Table 6).

Medication use

Regarding asthma medication use, at baseline, 53 out of 64 children were taking medication as part of their daily asthma therapy, of whom 51 children were taking anti-leukotriene agonists [intervention (24) versus control (27)]. At 6 months, 35 out of 64 children were taking medication [intervention (14) versus control (21)], with 31 children taking anti-leukotriene therapy (intervention (12) versus control (19). In the crude analysis, a greater reduction in anti-leukotriene use from baseline to 6 months was seen in the intervention group compared to the control group (24 versus 12 and 27 versus 19, respectively), although the result was not significant (χ^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 the present study is that fatty fish intake, specifically eating two meals of fatty fish (at least 150 g of 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 ATS guidelines, a reduction in FeNO by at least 10 units for values lower than 50 ppb as the cut-off point indicates 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 as to 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' [odds ratio (OR) = 0.62; 95% CI = 0.48–0.80] and 'current asthma' (OR = 0.75; 95%

Table 3 KIDMED Questionnaire baseline and 6-month follow-up

KIDMED Questionnaire		Baseline group			Six-month Group		
Question	Response	Intervention % (*n*)	Control % (*n*)	*P**	Intervention % (*n*)	Control % (*n*)	*P**
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 (40 g) 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*

Bold characters represent statistical significant *P*-values.
**P*-value calculated using the chi-squared test; *k*: constant.

Table 4 Plasma fatty acid composition of children at baseline and 6 months per group

Plasma fatty acids composition	Baseline group					Six-month Group				
	Intervention		Control			Intervention		Control		
Plasma fatty acid (μmol L^{-1})	Mean	(SD)	Mean	(SD)	$P^{\dagger}$	Mean	(SD)	Mean	(SD)	$P^{\dagger}$
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	1124.29	(443.25)	1147.32	(428.68)	0.87	1724.34	(546.64)	1602.04	(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	4939.01	(1084.76)	5118.12	(1175.32)	0.64	5973.66	(1391.99)	5678.64	(1229.67)	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	1557.90	(443.45)	1618.86	(479.28)	0.79	2254.36	(607.29)	2167.93	(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**

Bold characters represent statistical significant *P*-values.
†*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

	Group		Difference			
Mean change	Intervention	Control	Mean	95% CI	*P**	*P*†
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**

Bold characters represent statistical significant *P*-values.

**P*-value calculated using *t*-test.

†*P*-value from multiple linear regression analysis adjusted for confounders age, sex, body mass index and regular physical activity.

ACQ, asthma control questionnaire; CI, confidence interval; $FEF_{25–75\%}$, mid expiratory flow 25–75% vital capacity; FeNO, fractional exhaled nitric oxide analysis; FEV_1, forced expiratory volume in 1 s; FEV_1/FVC, ratio of forced expiratory volume and forced vital capacity; FVC, forced vital capacity; PAQLQ, paediatric asthma quality of life questionnaire; PEF, peak expiratory flow.

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

		Difference		
Mean score change	Variable	β	95% CI	*P**
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

Bold characters represent statistical significant *P*-values.

β, unstandardised beta; BMI, body mass index; CI, confidence interval; FeNO, fractional exhaled nitric oxide analysis.

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

CI = 0.60–0.95) in children up to 4.5 years old, whereas fatty fish intake was protective for 'current asthma' in children aged 8–14 years (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 warranting further exploration was that there was a higher reduction in medication use for children in the intervention group compared to the control group. 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, it was proposed that medication use could be decreased in some patients with asthma with an 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 the long-term intake of vegetable oils. However, the increase in tissue DHA may not be immediate and not as effective as the direct consumption of DHA from fish or fish oil supplements [17]. The Mediterranean dietary pattern is a varied diet that consists of a high intake of vegetables and wild edible greens, as well as a 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 is present in fatty fish. For example, Atlantic cod contains 277 mg of DHA per 180 g fillet, 29 mg in 180 g of chicken breast, 12 mg in an egg, 2 mg in a pork chop and 1 mg in 90 g of beef steak compared to 2477 mg in 180 g of farmed Atlantic salmon [49]. By 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 the consumption of fish leads to a shift in omega-3/omega-6 fatty acid balance resulting in a 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 also decrease pro-inflammatory mediators

from *n*-6 fatty acid arachidonic acid such as 2-series prostaglandins and 4-series leukotrienes (leukotriene E4, leukotriene B4), eosinophils and tumor necrosis factor-α, which promote airway oedema, mucus secretion, bronchial inflammation, bronchospasm and onset of asthma symptoms [19].The 2-series PG have an immunomodulatory function, which modifies the activity of macrophages and lymphocytes and suppresses the production of T-helper (Th)1-related cytokines, promoting the expression of the 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 immunoglobulin E via B lymphocytes and eosinophil production leading to severe bronchial inflammation and the onset of asthma symptoms [50,51]. Furthermore, EPA gives rise to eicosanoids with a lower biological potency than those generated from arachidonic acid [19] and thus they are weaker inducers of inflammation. Unique to EPA and DHA, both of these fatty acids are precursors to resolvins, and DHA is also a precursor to protectins and maresins, which are mediators with anti-inflammatory resolving properties. For example, the 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 concentration of IL-5, Th2 cytokines, Th17, airway mucus metaplasia as well as airway hyperactivity and promoted inactivity of pro-inflammatory transcription activator nuclear factor kappa B. 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 and reactive nitrogen species, 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 NO synthase, 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 the present study adds to the existing evidence. A strength of 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. By contrast, fish oil supplements might not provide sufficient anti-inflammatory activity because of impaired enzymatic activity in asthma patients [57]. Also, fish oil is known to have an unpleasant taste, odour and adverse effects such as gastrointestinal disturbances [58,59] and therefore is less palatable and not sustainable. Another strength of the present study is that pulmonary function and bronchial inflammation were assessed quantitatively compared to a parent's report of symptoms or the use of a questionnaire. Treatment and monitoring of asthma are guided by symptom scores or lung function parameters, which are not always accurate markers of disease severity [35]. Exhaled nitric oxide is an important biomarker for bronchial inflammation in asthma because a patient can be asymptomatic and spirometry may not always reflect the underlying inflammation [35]. Also, it is valuable for identifying eosinophilic inflammation, adherence and effectiveness of medication therapy and also for predicting the risk of future exacerbations [60]. In the present study, patient adherence was assessed by independent dietary biomarkers rather than using dietary data [46]. Furthermore, plasma fatty acid composition is a reliable indicator of dietary fat intake in children [61].

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

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MP is the principal author of the paper. CI is the principal investigator and DT, CK, MK, BE and MP are co-investigators. BE and KL assisted with the statistical analysis and supported the interpretation and revisions of the manuscript. All co-authors declare that they have seen and approved the final version of the manuscript submitted for publication.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Biochemical tests.

Figure S1. Fractional exhaled nitric oxide (FeNO) values from baseline to 6 months for participants in the intervention group.

