

Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children

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ABSTRACT: We assessed the clinical and biochemical effects in asthmatic children of fish oil supplementation and a diet that increases omega-3 and reduces omega-6 fatty acids.

Thirty nine asthmatic children aged 8–12 yrs participated in a double-blind, randomized, controlled trial for 6 months during which they received fish oil capsules plus canola oil and margarine (omega-3 group) or safflower oil capsules plus sunflower oil and margarine (omega-6 group). Plasma fatty acids, stimulated tumour necrosis factor α (TNF α) production, circulating eosinophil numbers and lung function were measured at baseline and after 3 and 6 months of dietary modification. Day and night symptoms, peak flow rates and medication use were recorded for 1 week prior to laboratory visits.

Plasma phospholipid omega-3 fatty acids were significantly greater in the omega-3 group at 3 and 6 months compared to the omega-6 group ($p < 0.001$). In the omega-3 group TNF α production fell significantly compared with baseline ($p = 0.026$), but the magnitude of change between groups did not reach significance ($p = 0.075$). There were no significant changes in clinical outcome measures.

Dietary enrichment of omega-3 fatty acids over 6 months increased plasma levels of these fatty acids, reduced stimulated tumour necrosis factor α production, but had no effect on the clinical severity of asthma in these children.

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We have previously shown that regular fish consumption [1] and particularly consumption of oily fish [2] is associated with reduced risk of children having asthma. This association led to the hypothesis that the ratio of omega-3 to omega-6 fatty acids in the diet may be one of the factors that can influence clinical severity of asthma [3].

Previous studies have shown that dietary supplementation with fish oil, a rich source of the omega-3 fatty acid, eicosapentaenoic acid (EPA), and/or one of the vegetable sources of omega-3 fatty acids, alpha linolenic acid (LNA), increases the levels of EPA in the phospholipids of cell membranes by up to 10 fold [4], and reduces the synthesis of the proinflammatory cytokines interleukin-1 and tumour necrosis factor (TNF) α in human mononuclear cells [5]. Although these changes have been associated with a reduction in the severity of late asthmatic responses to allergen [4], most of the clinical trials undertaken have shown no beneficial effect on the clinical severity of asthma. Generally, the studies have been short (8–10 weeks). Longer exposure periods may be required to reduce inflammation [6] and thus induce clinical improvement, although a study of 6 months duration showed no effect on the development of seasonal hay fever and asthma [7]. However, all of these studies were in adults whilst the epidemi-

ological studies showing reduced risk have been in children.

The aim of the present study was to explore a possible mechanism for the findings of these epidemiological studies through a randomized controlled trial in which the diets of asthmatic children were supplemented with either omega-3 or omega-6 fatty acids. The effects on clinical, biochemical and inflammatory parameters were measured over 6 months.

Methods

Subjects

Forty five asthmatic children, aged 8–12 yrs, with a history of episodic wheeze in the last 12 months and airway hyperresponsiveness (AHR) to histamine were recruited on a continuing basis over a period of 16 months. Six subjects dropped out at baseline. The children were randomly allocated to one of two diet groups: 20 in the omega-3 group (nine males, 11 females); and 19 in the omega-6 group (eight males, 11 females). Children with other significant diseases, taking regular oral corticosteroids or with

known aspirin or dietary salicylate sensitivity were excluded. The study was approved by the Ethics Review Committee of the Central Sydney Area Health Service and by the Human Ethics Committee of the University of Sydney.

Study design

There were two baseline visits separated by a 2 week run-in period, to establish the repeatability of the baseline measurements and to obtain diary card data of asthma severity. During the first baseline visit, responses to allergen skin prick tests and the child's history of respiratory symptoms and medication use as well as parental smoking, race and social class were documented. The second baseline visit was followed by 6 months on a fat modified diet. During the diet period, subjects took supplementary capsules containing either fish oil (omega-3 group) or safflower/palm/olive oil (omega-6 group) and were asked to use exclusively the margarines and oils supplied. Subjects were reviewed at 12 weeks and finally at 24 weeks from commencement of the dietary modification. At every visit AHR, height and weight were recorded and venous blood was collected for measurement of eosinophil levels, production of TNF α and the fatty acid composition of plasma. Severity of asthma was monitored via the completion of a parent supervised diary for 1 week prior to all but the first baseline visit. During the pre-visit week, peak flow readings, medication requirements and symptom scores were recorded daily. At the same time a detailed dietary record was kept.

Diets

Omega-3 diet. Canola oil and canola-based margarines and salad dressings (Meadowlea Pty Ltd) were supplied to the family in unmarked containers to replace their usual oils and margarines. Canola oil is high in α linolenic acid, an omega-3 fatty acid. Subjects were asked to use these oils and margarines exclusively and to have a meal containing fish at least once a month.

Omega-6 diet. Sunflower oil and sunflower oil-based margarines and salad dressings (Meadowlea Pty Ltd) were supplied in unmarked containers to the families. Sunflower oil is high in linoleic acid, an omega-6 fatty acid. Subjects were asked not to eat fish and to use the supplied oils and margarines exclusively.

Supplementary capsules. All subjects were asked to take four supplementary capsules per day. The omega-3 group took MaxEPA (R.P. Scherer, Melbourne, Australia) containing 0.18 g EPA and 0.12 g docosahexaenoic acid (DHA), to give a total of 1.2 g of omega-3 fatty acids per day. The omega-6 group took matched placebo capsules (R.P. Scherer), containing a combination of safflower (0.45 g), palm (0.45 g) and olive (0.1 g) oils per capsule. No EPA or DHA was present in the placebo preparation.

Subjects and laboratory staff were blinded to the study groups. Compliance with taking the supplementary capsules was assessed by counting the number of unused capsules.

Diet diary

A detailed diary of all types and household measures of food and drinks consumed, including brand names, was kept for 1 week during the baseline and after 3 and 6 months of dietary modification and supplementation. Data from the diary was used to check compliance with the diet and that dietary intakes did not alter more than would be expected over the 6 months of study, taking into consideration the growth of the children.

Lung function and AHR

Lung function and AHR were measured at the beginning and end of baseline and after 3 and 6 months of treatment. A Vitalograph wedge bellows spirometer (Vitalograph Ltd, Bucks, UK) was used to measure forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁). The highest of two values for FEV₁ repeatable to within 100 mL was recorded and the percentage of predicted values [8] was calculated.

Airway responsiveness was measured using histamine inhalation tests performed according to the method of YAN *et al.* [9]. Briefly, histamine was administered to the subject *via* hand-held DeVilbiss No 45 plastic nebulizers (DeVilbiss Health Care Inc, Somerset, PA, USA) in doubling doses, ranging 0.03–7.8 μ mol, until the FEV₁ fell by 20% or more. The dose of histamine causing the maximum fall in FEV₁ was used to calculate the dose response ratio, which is the percentage fall in FEV₁ divided by the cumulative dose of histamine [10]. All short acting aerosol bronchodilators were withheld for 6 h and long acting bronchodilators for 36 h.

Atopy

Atopy was defined as a mean wheel size of ≥ 3 mm to at least one of 13 allergens applied with skin prick to the forearm (house dust, *Dermatophagoides pteronyssinus*, *D. farinae*, cat hair and dander, dog hair and dander, horse, mixed feather, ragweed, plantain, timothy grass, rye-grass, *Aspergillus fumigatus* and *Alternaria tenuis*).

Asthma severity

Asthma severity was measured for 1 week during the week prior to the second laboratory visit at baseline, and after 3 and 6 months of the fat modified diet. Asthma severity was measured using a composite severity score based on daily diary records of expiratory flow rate, day and night symptoms and medication use. Each component of the score contributes up to four points to a maximum composite score of 16. Expiratory flow rate was measured on waking, before bronchodilator, using an Assess peak flow meter (HealthScan Products, NJ, USA) or a Mini Wright peak flow meter (Clement Clarke International Ltd, Essex, UK) and scored according to the percentage of the predicted value. Medication use was scored according to the frequency and type of medication. Symptom scores for wheeze, cough and shortness of breath were recorded for both day and night, with scores ranging from 0 (no symptoms) to 4 (symptoms that make normal activity, or sleeping, impossible).

Blood analysis

Venous blood (20 mL) was collected at each laboratory visit. Full blood counts were performed on every occasion by automated full blood count analyser (Bayer Technicon H2, Tarrytown, CT, USA). Peripheral blood mononuclear cells (PBMC) were purified from anti-coagulated peripheral blood by discontinuous density gradient centrifugation using mono-poly resolving medium (ICN Biomedicals, Costa Mesa, USA) [11] and cultured in RPMI-1640 supplemented with Monomed A (CSL Ltd., Victoria, Australia) at 2×10^6 cells·mL⁻¹ in a 5% CO₂ in air atmosphere at 37°C. PBMC cultures were activated with lipopolysaccharide (10 ng·mL⁻¹), harvested and stored at -80°C. Total PBMC synthesis of TNF α was assessed in the cell cultures after freeze-thawing [5] using a sandwich enzyme-linked immunosorbent assay (ELISA) and the human TNF α DuoSet™ system (Genzyme Diagnostics, Cambridge, MA, USA).

For fatty acid analysis, plasma lipids were extracted by the method of BLIGH and DYER [12]. Phospholipids were separated from neutral lipids using a silica column. Tubes containing phospholipids were flushed with nitrogen and stored at -80°C until fatty acid determination. The phospholipids were transesterified using a one-step methanolysis reaction [13] and fatty acids analysed using flame ionisation capillary gas chromatography (Capillary Gas Chromatograph; Hewlett-Packard, North Ryde, NSW, Australia) using a fused carbon-silica column, coated with cyanopropylphenyl (J and W Scientific, Folsom, CA, USA), hydrogen carrier gas and two step oven temperature programme to allow optimal separation. Individual fatty acids (including linoleic acid, arachidonic acid, EPA, docosapentaenoic acid, DHA) were identified by comparison with a standard mixture (Nu Check Prep, Elysian, MN, USA) to which EPA (Sigma, St Louis, MO, USA) was added. The fatty acids were expressed as a percentage of total fatty acids.

Statistical analysis

Data are reported as mean \pm 95% confidence interval (95% CI). Since there were no significant differences between the first and second run-in values for any variable, baseline values were calculated as the mean of the two run-in values. Differences between groups were determined by analysis of variance (ANOVA) for repeated measures, for continuous variables, and by Chi-squared test for categorical variables. In addition, mean changes from baseline to 3 months and from baseline to 6 months were calculated and compared between groups by t-test. Values for dose response ratio were log transformed before analysis. Median values for eosinophil numbers and asthma severity scores were estimated and changes from baseline compared at 3 and 6 months by Mann-Whitney test.

Results

Baseline characteristics of the two groups are shown in table 1. At baseline there were no significant differences between the groups in lung function, expressed as a percentage of the predicted value, AHR, atopy, use of inhaled corticosteroids, plasma fatty acid levels, eosinophil levels

Table 1. – Baseline details of the two subject groups

	Omega-6 group	Omega-3 group
Subjects n	19	20
Females/males	11/8	12/8
Age yrs	9.7 \pm 0.4**	10.8 \pm 0.6
Height cm	139 \pm 4**	146 \pm 4
Atopic n	17	19
Median asthma score	8.0 (7.0–9.5)	8.0 (5.5–9.5)
FEV ₁ % pred	86.1 \pm 7.0	81.1 \pm 5.8
FVC % pred	87.4 \pm 6.2	85.8 \pm 5.5
Dose response ratio	30.8 (16.7–56.7)	37.3 (18.8–74.3)
Medication n		
Inhaled steroids	13	13
β agonists	15	20
Cromoglycate	6	4
Plasma omega-3 % total fatty acids	2.26 \pm 0.22	2.14 \pm 0.37
Plasma EPA % total fatty acids	0.54 \pm 0.09	0.45 \pm 0.11
TNF α production pg·mL ⁻¹	1284 \pm 230	1300 \pm 316
Eosinophils $\times 10^9$ ·L ⁻¹	0.62 (0.55–0.93)	0.91 (0.53–1.18)

Values are presented as mean \pm 95% confidence interval, with the exception of asthma severity score and eosinophil number, which are presented as median and interquartile range. **: p<0.01, versus Omega-3 group. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; EPA: eicosapentaenoic acid; TNF α : tumour necrosis factor α .

or mononuclear TNF α production. The omega-3 group was slightly older than the omega-6 group (10.8 \pm 0.6 yrs and 9.7 \pm 0.4 yrs, respectively; p<0.01).

There was no significant difference between groups in the quantity of oil and margarine consumed throughout the study. The omega-3 group ate significantly more fresh fish than the omega-6 group (370 \pm 148 and 109 \pm 70 g·month⁻¹, respectively; p=0.0045). The mean number of capsules taken per day was three out of a recommended four in both groups, but no child averaged less than two capsules per day. One child in the omega-3 group and two children in the omega-6 group experienced some discomfort after taking the capsules which was not related to their asthma. Medication use did not change significantly throughout the study.

Fatty acids

The mean change in plasma phospholipid omega-3 fatty acids, as a percentage of total fatty acids, was significantly greater in the omega-3 group at 3 and 6 months than in the omega-6 group (mean change at 3 months 3.18 \pm 0.88% versus -0.21 \pm 0.24%, respectively, p<0.001; mean change at 6 months 2.19 \pm 0.67% versus 0.05 \pm 0.41%, respectively, p<0.001). The mean change in plasma EPA was also significantly greater in the omega-3 group at 3 and 6 months compared to the omega-6 group (mean change at 3 months 1.98 \pm 0.53% versus -0.11 \pm 0.09%, respectively, p<0.001; mean change at 6 months 1.2 \pm 0.42% versus 0.19 \pm 0.44%, respectively, p=0.0024) (fig. 1).

Lung function and asthma severity

There was no significant change in spirometric function, dose response ratio to histamine or asthma severity scores

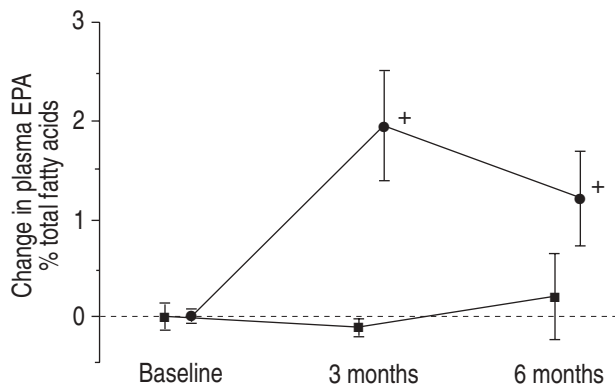


Fig. 1. – Change in plasma eicosapentaenoic acid (EPA) in children in the omega-6 (■) and the omega-3 (●) groups. The errors bars at baseline show 95% confidence intervals (CIs) of the mean difference of the two baseline readings. Values shown at 3 and 6 months are the mean differences from baseline \pm 95% CIs. +: $p < 0.001$, versus omega-6 group.

at either 3 months or 6 months in either group. Mean FEV₁ at 6 months was $83.7 \pm 5.3\%$ pred in the omega-3 group and $83.5 \pm 5.2\%$ pred in the omega-6 group, while median asthma severity score was 7 in the omega-3 group and 8 in the omega-6 group. The mean change in dose response ratio at 6 months was -0.38 ± 0.64 doubling doses in the omega-3 group and 0.54 ± 0.90 doubling doses in the omega-6 group ($p = 0.10$).

TNF α

In the omega-3 group TNF α production from stimulated PBMCs fell significantly over the 6 month period from $1,300 \pm 316$ to 896 ± 21 pg·mL⁻¹ ($p = 0.026$), but the magnitude of the changes did not differ significantly between groups (mean change at 6 months was -416 ± 331 pg·mL⁻¹ in the omega-3 group and 44 ± 359 pg·mL⁻¹ in the omega-6 group; $p = 0.075$) (fig. 2). There were no significant differences between groups in TNF α production over time ($p = 0.22$ using repeated measures ANOVA).

Peripheral blood eosinophils

In the omega-3 group, eosinophil numbers fell throughout the study from a median value of 0.91×10^9 cells·L⁻¹ at baseline, to 0.74×10^9 cells·L⁻¹ at 3 months and 0.65×10^9 cells·L⁻¹

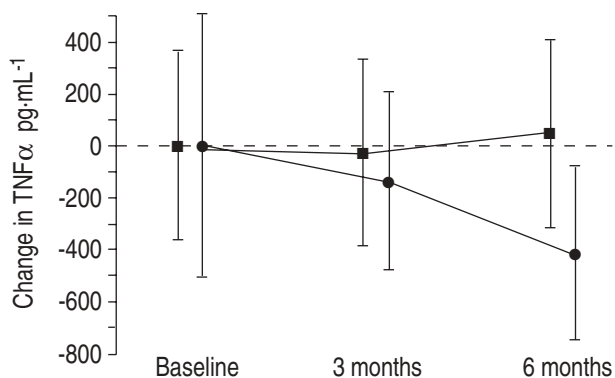


Fig. 2. – Change in plasma stimulated tumour necrosis factor α (TNF α) production in children in the omega-6 (■) and the omega-3 (●) groups. The errors bars at baseline show 95% confidence intervals (CIs) of the mean difference of the two baseline readings. Values shown at 3 months and 6 months are the mean differences from baseline \pm 95% CIs.

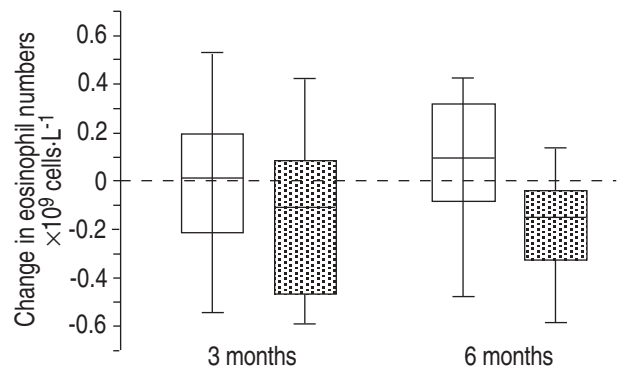


Fig. 3. – Change in peripheral blood eosinophilia in children in the omega-6 (□) and omega-3 (▨) groups. The unbroken line within the box marks the median, the lower boundary of the box indicates the 25th percentile and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles.

L⁻¹ at 6 months. In the omega-6 group eosinophil numbers rose from a median value of 0.62×10^9 cells·L⁻¹ at baseline, to 0.70×10^9 cells·L⁻¹ at 3 months and 0.81×10^9 cells·L⁻¹ at 6 months. However, the magnitude of these changes did not differ significantly between groups ($p = 0.11$, Mann-Whitney test) (fig. 3).

Discussion

This study has shown that, in asthmatic children, a modest fish oil supplement of 1.2 g·day⁻¹ of omega-3 fatty acids and relatively minor changes to the diet caused a fivefold increase in plasma EPA. Although there were no significant clinical effects, there was a trend towards reduced TNF α production and reduced numbers of circulating eosinophils. The effect of fatty acid intake on circulating eosinophil numbers and TNF α production in asthmatic children has not been examined in any previous study.

Sample size calculations for this study were based on changes in AHR as the principal outcome variable, and the study would have been able to detect a difference between groups of one doubling dose change. In the absence of any prior data on TNF α production in asthmatic children, no sample size calculations for the effect on TNF α were possible. However, data from the present study show that the power of the study to detect a significant effect on TNF α production was 71%, suggesting that, with a larger sample size, significant changes in TNF α production may have been detected.

The changes in omega-3 fatty acids in plasma phospholipids achieved in this study are similar to studies that used much larger supplements of fish oil (10 – 15 g·day⁻¹) [14] suggesting that significant omega-3 incorporation into phospholipids can be attained with relatively low doses of fish oil. Changes of this magnitude have been associated with significant reductions in neutrophil chemotaxis [15]. The observed changes in plasma EPA levels confirm that compliance was good over the whole period of the trial.

TNF α production is implicated in the pathogenesis of asthma since it is increased in asthmatics [16] and increases airway responsiveness both *in vitro* [17] and *in vivo* [18]. The effect of fatty acid intake on TNF α production has been well established and it has been shown that significant reductions in stimulated TNF α production can

be achieved with smaller numbers of subjects and shorter duration of the study period than the present study [5, 19]. However, the subjects of these studies were healthy male adults. It is possible that any changes in an asthmatic subject would be slower due to the presence of active inflammation. There was a downward trend in both TNF α production and circulating eosinophil numbers which was consistent at both 3 and 6 months, suggesting that a study of longer duration might have produced more significant effects.

Changing the fatty acid intake had no effect on any clinical measure of asthma severity in these asthmatic children. This finding is in accordance with those of previous studies in adult asthmatics [4, 6, 7], and suggests that, in children with existing asthma, modification of fat intake is unlikely to have any short-term therapeutic benefit. In the absence of any therapeutic effect, a number of plausible mechanisms may explain the observed reduction in the risk of having asthma in children who eat oily fish. If the diet measured by a food frequency questionnaire is typical of lifetime dietary habits of children and parents, it is possible that a diet rich in omega-3 fatty acids during early life, or even prenatally, may prevent the development of asthma by reducing inflammatory responses to allergens in susceptible individuals [20]. Alternatively, modest differences in dietary fats over a longer period of time may modify cytokine production and inflammatory processes, potentially reducing symptoms in the long term.

Circulating eosinophil numbers and TNF α production continued to fall at 6 months in the present study even though changes in omega-3 fatty acids in the plasma phospholipids were maximal at 3 months. How long they would continue to fall if the increased level of omega-3 fatty acids was maintained is unknown. Presumably, these changes indicate a reduction in airway inflammation, but whether fish oil could suppress the production of cytokines and eosinophils to levels similar to those attained with appropriate corticosteroid therapy, which is associated with clinical improvement, is also unknown. Clinical experience with rigorous corticosteroid therapy suggests that inflammation may need to be suppressed for prolonged periods (years) in order to reverse the structural changes characteristic of asthmatic airways.

We conclude that a fish oil supplement along with the addition to the diet of canola oil and canola oil margarine over 6 months increased the plasma levels of omega-3 fatty acids, but had no effect on the clinical severity of asthma in these children. However, there was a downward trend in both eosinophil numbers and tumour necrosis factor α production in the omega-3 group. This suggests that increases in dietary omega-3 fatty acids over a longer period of time, say years, may be required to reduce the severity of existing asthma. It is yet to be determined if increasing dietary omega-3 fatty acid intake in early life can prevent the development of asthma.

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