

Effect of omega-3 fatty acid concentrations in plasma on symptoms of asthma at 18 months of age

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The objective of this study was to assess the relation between observed levels of omega-3 fatty acids in plasma and symptoms of asthma and atopy in children at 18 months of age. A total of 616 women at risk of having a child who would develop asthma because of a family history were recruited from the antenatal clinics of six hospitals in Sydney, Australia. Families were randomized to either active omega-3 supplemented or control group. The active group received a daily tuna fish oil supplement and omega-3-rich margarines and cooking oils and the control group received a placebo supplement with polyunsaturated margarines and cooking oils. When the children were 18 months of age an assessment of symptoms was carried out by a research nurse blinded to treatment group allocation. Atopy was measured by skin prick tests, blood was collected to determine serum immunoglobulin E (IgE), and plasma fatty acid concentrations. A total of 376 children (61.0% of total recruited) completed an assessment at 18 months and had blood taken to determine plasma fatty acid concentrations. Omega-3 fatty acid levels were expressed in quintiles of exposure 'as treated' without reference to treatment group allocation. Wheeze ever, doctor visits for wheeze, bronchodilator use and nocturnal coughing were significantly reduced in children in the higher exposure quintiles. Serum IgE was reduced in the highest quintile but not significantly so. There was no difference in diagnosed asthma or atopy between the exposure quintiles. Although wheeze at this age may not be a good indicator of asthma in later childhood, it is encouraging that some symptoms have been reduced in children with high omega-3 fatty acid concentrations in plasma.

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The omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high proportions in oily fish. These fatty acids have anti-inflammatory effects and high dietary levels are associated with a lower incidence of inflammatory diseases such as rheumatoid arthritis (1, 2). Some clinical studies have reported that oral supplementation with fish oil has beneficial effects on patients with asthma (3–6), however, others have reported no benefit (7–10). A recent meta-analysis concluded that there was little evidence to recommend that people with asthma supplement or modify their diet

to increase intake of omega-3 fatty acids in order to improve their symptoms (11).

Epidemiological studies in Australia have reported that children who have a greater consumption of oily fish have a significantly reduced risk of current asthma (12). To date there has been no study to test the benefits of fish oil for the primary prevention of asthma in young children. The CAPS study is a randomized-controlled trial of fish oil supplementation and house dust mite avoidance for the primary prevention of asthma in children (13). The active dietary intervention comprises the use of a daily fish oil supplement

from 6 months of age or at onset of bottle-feeding, and use of margarine and cooking oils based on canola oil to increase omega-3 dietary intake and limit omega-6 fatty acids. We have recently reported the initial results by intention-to-treat and showed that the dietary intervention resulted in a reduced prevalence of wheeze ever and wheeze for >1 wk (14). Here, we report the 'as treated' analysis, i.e. the relation between observed levels of omega-3 fatty acids in plasma and symptoms of asthma and atopy in children at 18 months of age in order to assess whether there is a dose-dependent relationship.

Methods

The details of the study design and recruitment procedure have been reported (13) and are briefly described here.

Participants

A total of 616 pregnant women whose unborn children were at high risk of developing asthma because of a parent or a sibling with a current diagnosis of asthma or with frequent wheeze were recruited from the antenatal clinics of six hospitals in Sydney, Australia. Women were randomized before birth of the child into the diet active intervention or the diet control intervention. Children were visited at home at age 1, 3, 6, 9, 12 and 18 months to monitor adherence with the intervention, growth and collect symptom data.

Ethics approvals

The study was approved by ethics committees of the University of Sydney, the Children's Hospital at Westmead, and the Western and South Western Sydney Area Health Services.

Intervention

Families in the dietary intervention groups were provided with a daily omega-3 fatty acid-rich tuna fish oil supplement (500 mg; Clover Corporation, Sydney, Australia), which had a slight fishy taste and smell. Parents received the instruction that the child was to be given the supplement from the age of 6 months. If the child was breast-feeding before 6 months of age no supplement was given, because the concentration of omega-3 fatty acids in breast-milk was equivalent to that of the supplement. If bottle-feeding was introduced before the age of 6 months parents were asked to add the oil supplement to the child's formula. The dose was standardized

according to the fluid intake and hence the age of the child. In addition, each family was provided with canola-based oils and spreads, which are rich in omega-3 fatty acids, for use in food preparation. The intention was to replace polyunsaturated oils and spreads, which are relatively high in omega-6 fatty acids with oils and spreads rich in omega-3 fatty acids. The control group received placebo supplements made up of Sunola oil, a mostly monounsaturated oil (Clover Corporation) and were provided with polyunsaturated oils and margarines for use in food preparation. Adherence with the interventions was assessed at each visit with the use of a questionnaire that included both self-reported and nurses' rating of compliance.

Outcomes

At age 18 months each child had a clinical assessment performed by a nurse who was blinded to treatment group allocation. This assessment included an interviewer-administered questionnaire designed to collect information on symptoms, diagnoses and treatment of asthma, and other allergic illness. Where possible questions were taken from standard questionnaires, which were validated for use in young children. Clinical procedures that the nurse performed included an allergen skin prick test and blood collection for total serum immunoglobulin E (IgE), and plasma fatty acids. All laboratory analyses were undertaken by research assistants who were blinded to treatment group allocation.

Skin prick tests

Allergy was assessed using skin prick tests to six ingested allergens (salmon, tuna, peanuts, cows milk and egg) and five inhaled allergens (house dust mite, grasses, cockroach, cat, *Alternaria*). Glycerine and histamine (6 mg/ml) were used as negative and positive controls, respectively. Any allergen wheal size ≥ 2 mm and larger than the negative control was regarded as positive. Where there were no positive allergen wheals and the histamine wheal was also negative, the test was repeated.

Fatty acid analysis

Plasma lipids were extracted from blood samples with chloroform:methanol (15). Plasma phospholipids were separated by thin layer chromatography and evaporated to dryness under nitrogen. The samples were methylated in 1% H₂SO₄ in methanol at 70°C for 3 h. When

cooled, the resulting methyl esters were extracted into *n*-heptane and transferred to vials containing anhydrous Na₂SO₄ as the dehydrating agent.

Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Littleforth, DE, USA) equipped with a 50 m capillary column (0.33 mm ID) coated with BPX-70 (0.25 µm film thickness SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250°C and the detector (flame ionization) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C/min. Helium was used as the carrier gas at a velocity of 35 cm/s. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards obtained from Nuchek Prep Inc. (Elysian, MN, USA). The proportion of total fatty acids that were omega-3 and omega-6, respectively, were calculated for each subject in grams and expressed as a percentage of the total fatty acids.

Blinding

The use of placebo supplements, oils and margarines helped to ensure single blinding for the diet intervention. However, the slight fishy smell of the active supplements meant that some participants in the intervention groups might have been aware of the supplement constituency. For both groups, supplements were described as natural food oil supplements, therefore, the participants were not aware of whether they were in the active or control. Because information about compliance was collected in each group, we were able to detect differences in the supplement use between groups.

For this study, it was not possible to blind the research nurses to group allocation because they were responsible for explaining the study and giving different advice to each group. However, the research nurses who undertook the outcome assessments and the statistician who conducted the analyses were blinded to the group allocation of the participants.

Statistical methods

Children were divided into groups according to quintile of plasma omega-3 fatty acid concentration. Chi-square and chi-square trend tests were used to assess the difference between categorical outcomes. Geometric mean values for IgE were calculated for each quintile of omega-3 fatty acid concentration and analysed using one-way ANOVA. Logistic regression was used to adjust for confounders in the analysis.

Results

A total of 376 (61.0%) children of the 616 enrolled in the study completed the 18 month assessment and had blood taken to determine plasma fatty acid concentration. Sixty-two children were withdrawn from the study before 18 months of age and the remaining 178 did not have blood taken.

Table 1 shows there was no effect of high omega-3 fatty acid concentrations in plasma on prevalence of diagnosed asthma or atopy. However, wheeze ever, doctor visits for wheeze, bronchodilator use and nocturnal coughing were significantly reduced in the higher exposure quintiles. Serum IgE was reduced in the highest quintile but not significantly so. All other outcomes related to early symptoms of asthma did not show a significant trend across quintiles.

The prevalence of risk/protective factors for asthma including breast-feeding ever, maternal and paternal asthma, male gender, parity and exposure to tobacco smoke during childhood were equally distributed between the quintiles. However, as shown in Fig. 1, maternal smoking during pregnancy was significantly lower in the highest quintiles (chi-squared trend $p = 0.014$) and duration of breast-feeding was significantly higher in the highest quintiles (chi-squared trend $p = 0.003$).

Children whose mothers smoked during pregnancy were more likely to wheeze (57.3% vs. 42.5%, $p = 0.02$) and visit the doctor for wheeze (32.9% vs. 21.4%, $p = 0.04$) than children with mothers who did not smoke during pregnancy. However, children with mothers who smoked were also less likely to be atopic (9.6% vs. 21.7%, $p = 0.01$). After adjusting for smoking in pregnancy (OR = 1.39, 95% CI: 0.71–1.95) and breast-feeding for more than 1 month (OR = 0.72, 95% CI: 0.45–1.14) being in the highest exposure quintiles was still a significant protective factor for bronchodilator use (OR = 0.46, 95% CI: 0.30–0.71, $p < 0.001$).

There were no differences in any of the outcome variables between children who did or did not have blood collected and protective/risk factors for asthma (such as breast-feeding, smoking and gender) were also equally distributed in these groups.

Discussion

Although we did not find a dose-dependant relationship, we found that higher levels of omega-3 fatty acids in plasma were associated with a reduced prevalence of some asthma

Table 1. Outcome by quintile of omega-3 fatty acid concentration in plasma

Quintile	Mean of total	Lowest		Middle		Highest	p-Value	Trend p-value
		1	2	3	4	5		
Total number (N)		75	76	76	76	75		
Omega-3 fatty acid in percentage of total fatty acids (95% CI)	5.85 (5.67–6.03)	2.14–4.40	4.41–5.02	5.02–5.97	5.98–7.26	7.29–11.71		
Outcomes								
Serum IgE geometric mean (IU/l, 95% CI)	20.31 (17.62–23.42)	20.4 (14.5–29.1)	19.3 (13.9–26.7)	22.1 (15.9–30.6)	22.8 (16.7–31.2)	16.9 (12.5–23.0)	0.713	–
Atopy (SPT-positive, %)	19.0	17.3	15.8	21.1	19.7	21.3	0.888	0.404
Cough (>1 wk, %)	46.8	46.7	57.3	41.3	41.9	46.7	0.291	0.399
Cough during sleep (%)	6.7	12.0	14.7	2.7	4.1	6.7	0.029	0.032
Wheeze ever (%)	45.7	60.0	41.3	46.7	39.5	41.3	0.073	0.031
Wheeze (>1 wk, %)	25.8	37.3	24.0	24.0	17.1	26.7	0.075	0.077
Wheeze with difficulty breathing (%)	17.0	20.0	18.7	16.0	9.2	21.3	0.293	0.616
Doctors visit for wheeze (%)	23.9	33.3	25.3	22.7	15.8	22.7	0.157	0.047
Asthma diagnosed by a doctor (%)	12.7	18.7	11.8	11.8	5.3	16.0	0.134	0.325
Bronchodilator use (%)	45.8	64.0	47.4	50.0	30.3	37.3	<0.001	<0.001
Inhaled steroid use (%)	15.1	21.3	15.8	13.2	7.9	17.3	0.207	0.222
Eczema reported (%)	28.5	24.0	34.2	26.7	24.3	33.3	0.466	0.596
Eczema diagnosed by doctor (%)	29.1	24.0	31.6	32.9	25.0	32.0	0.622	0.575

CI, confidence interval; IgE, immunoglobulin E; SPT, skin prick test.

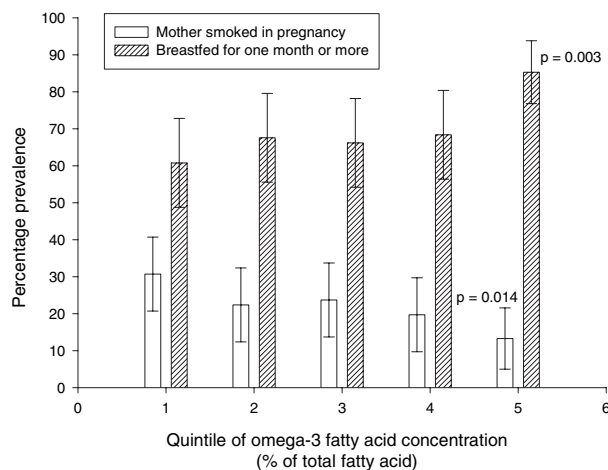


Fig. 1. Breast-feeding and smoking prevalence in each quintile of omega-3 fatty acid exposure.

symptoms. Children with high levels of omega-3 fatty acids in their plasma at age 18 months were less likely to have had wheeze or visited the doctor for wheeze but there was no similar trend for doctor diagnosed asthma. Children with higher levels of omega-3 fatty acids in plasma were less likely to have nocturnal coughing or use a bronchodilator. Although, mothers with children in the highest quintiles were more likely to have breast-fed their children and were less likely

to have smoked during their pregnancy, this result remained after adjusting for these factors.

Maternal smoking and exposure to tobacco smoke during pregnancy are known to increase the risk of wheezing illness in early childhood (16). It is possible that women who had better health conscious and therefore less likely to smoke and this in turn had an effect on early asthma symptoms in their children. There was no significant imbalance of the other confounders for asthma including gender, parity, maternal and paternal asthma, between quintiles. However, there may have been an imbalance of possible unknown confounders in our exposure groups.

Only 61% of children in the study had plasma fatty acid concentrations determined. The lack of complete information is a possible source of bias in our study. Apart from loss to follow-up, only 68% of children who had assessment data available had blood taken. It is possible that the children who did not have blood taken were different in terms of their plasma fatty acid concentrations than children who did although there were no differences in asthma symptoms or demographic factors between the group who had blood collected and the group who did not.

For other important indicators of early asthma, such as wheeze in the absence of a cold and

wheeze causing difficulty breathing, we found no significant reduction in prevalence in groups with high levels of plasma omega-3 fatty acid. Also, there was no significant trend in the prevalence of atopy although serum IgE was lower in the children in the highest quintile.

Previous studies conducted to measure the effects of omega-3 fatty acid supplementation on children and adults with asthma have shown little or no effect on symptoms. This may be because already established asthma is an irreversible condition. No studies to date have been designed to measure the effects of omega-3 fatty acids supplementation on the primary prevention of asthma in a large cohort of children. The mechanism of action of omega-3 fatty acids in reducing inflammation is that they competitively inhibit the formation of leukotrienes and prostoglandins from arachidonic acids and omega-6 fatty acids and reduce the generation of cytokines from inflammatory cells (17). It is biologically plausible, therefore, that children who have high amounts of omega-3 fatty acid in their plasma will have less airway inflammation and therefore fewer symptoms of asthma.

Although symptoms of wheeze at this age may not be a good indicator of asthma in later childhood, it is encouraging that some symptoms have been reduced in children with high omega-3 concentrations in plasma. Our results suggest that there is no dose-response relationship, but that there may be a threshold effect. Important markers of asthma severity such as bronchodilator use were reduced at this early age and thus the role of omega-3 fatty acid supplementation in the primary prevention of asthma cannot be discounted. We will assess the final outcome of our dietary intervention when the children are 5 yr old and their asthmatic and allergic status can be more reliably characterized.

Although this analysis is not as rigorous as the analysis by intention-to-treat, we wanted to determine whether a dose-dependent relationship existed between omega-3 fatty acid levels and symptoms. The associations observed in this analysis support the conclusions of the primary intention-to-treat analysis that omega-3 supplementation during the first 18 months of life does have beneficial effects in reducing the occurrence of wheezing illness during infancy.

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