

Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: A randomized, controlled trial

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Background: There is growing interest in the potential role of anti-inflammatory n-3 polyunsaturated fatty acids (n-3 PUFAs) in the prevention of allergic disease.

Objective: We sought to determine whether maternal dietary supplementation with n-3 PUFAs during pregnancy could modify immune responses in infants.

Methods: In a randomized, controlled trial 98 atopic, pregnant women received fish oil (3.7 g n-3 PUFAs per day) or placebo from 20 weeks' gestation until delivery. Neonatal PUFA levels and immunologic response to allergens were measured at birth. **Results:** Eighty-three women completed the study. Fish oil supplementation (n = 40) achieved significantly higher proportions of n-3 PUFAs in neonatal erythrocyte membranes (mean $\pm$ SD, 17.75% $\pm$ 1.85% as a percentage of total fatty acids) compared with the control group (n = 43, 13.69% $\pm$ 1.22%, $P < .001$). All neonatal cytokine (IL-5, IL-13, IL-10, and IFN- γ) responses (to all allergens) tended to be lower in the fish oil group (statistically significant only for IL-10 in response to cat). Although this study was not designed to examine clinical effects, we noted that infants in the fish oil group were 3 times less likely to have a positive skin prick test to egg at 1 year of age (odds ratio, 0.34; 95% confidence interval, 0.11 to 1.02; $P = .055$). Although there was no difference in the frequency of atopic dermatitis at 1 year of age, infants in the fish oil group also had significantly less severe disease (odds ratio, 0.09; 95% confidence interval, 0.01 to 0.94; $P = .045$).

Conclusions: These data suggest a potential reduction in subsequent infant allergy after maternal PUFA supplementation. More detailed follow-up studies are required in larger cohorts to establish the robustness of these findings and to ascertain their significance in relation to longer-term modification of allergic disease in children. (*J Allergy Clin Immunol* 2003;112:1178-84.)

Key words: Long-chain polyunsaturated fatty acids, cord blood, T_H2 cytokines, allergens, allergy, infants, docosahexaenoic acid, fish oil, pregnancy, cytokines

The dramatic increase in the expression of asthma and allergic disease over the last 20 to 30 years has highlighted the urgent need to identify associated factors that might be logical targets for disease prevention. One significant change during this period has been a progressive decline in the intake of dietary anti-inflammatory "n-3" polyunsaturated fats (n-3 PUFAs) in Western diets, with a corresponding increase in n-6 PUFAs.¹ Both epidemiologic and experimental data also provide a plausible link between these dietary changes and allergic diseases such as asthma.²⁻⁴ Although trials with n-3 PUFA supplementation in adults with established asthma have generally been disappointing,⁵ this might indicate that once allergic immune responses are established, this kind of intervention could be too late. Since events initiating allergic immune responses might begin before birth,⁶ we speculated that supplementation with anti-inflammatory n-3 PUFAs (fish oil) in pregnancy might provide a noninvasive intervention to modify immune development before disease is established. To address this, we performed a randomized, controlled trial first, to determine the feasibility, safety, and effectiveness of maternal fish oil supplementation in modifying neonatal n-3 PUFA status. Second, we assessed the effects on neonatal immune responses and the development of subsequent allergic symptoms and sensitization at 1 year of age.

METHODS

Study design

Allergen-specific T-cell responses in cord blood were compared in fish-oil-supplemented and control groups in a double-blind, placebo-controlled study. The number of subjects in this study was based on previous studies by the host laboratory comparing cord blood immune responses of neonates with or without later allergy. Power calculations determined that our sample size was sufficient for the detection of a difference of 15% in cytokine levels between control and intervention groups at a significance level of $P = .05$. The study was not designed to detect differences in clinical outcome measures, because a much larger population would be needed to address this issue. However, clinical outcome at 1 year has been reported as a secondary outcome measure.

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Abbreviations used

AA: Arachidonic acid (20:4n-6)
CBMC: Cord blood mononuclear cell
DHA: Docosahexaenoic acid (22:6n-3)
EPA: Eicosapentaenoic acid (20:5n-3)
HDM: House dust mite
n-3 PUFAs: Omega-3 polyunsaturated fatty acids
n-6 PUFAs: Omega-6 polyunsaturated fatty acids
OR: Odds ratio

Participants

Ninety-eight pregnant, atopic women booked for delivery at St John of God Hospital, Subiaco, Western Australia, between January 1999 and September 2001 were recruited into the study because their offspring were considered to be at high risk of allergic disease. All women had a history of physician-diagnosed allergic rhinitis and/or asthma and 1 or more positive skin prick tests to common allergens (house dust mite; grass pollens; molds; and cat, dog, and cockroach extracts; Hollister-Stier Laboratories, Spokane, Wash). Women were ineligible for the study if they smoked; if they had other medical problems, complicated pregnancies, or seafood allergy; or if their normal dietary intake exceeded 2 meals of fish per week. At 20 and 30 weeks' gestation, the women completed a validated, semiquantitative food frequency questionnaire,⁷ which was used to identify any background dietary change in fish consumption in each group. Pregnancy outcomes were assessed using data from all participants. The trial protocol was approved by the Ethics Committees at St John of God Hospital and Princess Margaret Hospital, and all women gave informed consent.

Assignment

The groups were block-randomized according to parity (no previous term childbirth versus 1 or more), prepregnancy body mass index (BMI), age, and maternal allergy (allergic rhinitis or asthma). Women in the fish oil group received 4 (1-g) fish oil capsules per day (Ocean Nutrition, Halifax, Nova Scotia, Canada) comprising a total of 3.7 g of n-3 PUFAs with 56.0% as docosahexaenoic acid (DHA) and 27.7% as eicosapentaenoic acid (EPA) (confirmed by gas chromatography). The control group received 4 (1-g) capsules of olive oil per day (containing 66.6% n-9 oleic acid and <1% n-3 PUFAs; Pan Laboratories, Moorebank, NSW, Australia). Compliance was monitored by measuring the incorporation of DHA and EPA into the cell membranes of erythrocytes.

Masking

Randomization and allocation of capsules occurred at a different center separate from the recruitment of participants. Capsules were administered to the participants by someone separate from those doing the allocation. The capsules in the 2 groups were image-matched, and the participants, research scientists, and pediatrician remained blinded to the groups for the duration of the study.

Fatty acid analyses

Phospholipid fatty acid analyses were carried out as previously described.⁸ In brief, total lipids were extracted from red blood cell membranes with methanol and chloroform. The phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography, and the fatty acid methyl esters were analyzed by gas-liquid chromatography. The fatty acids were expressed as a percentage of the weight of the total fatty acids measured (C14 to C22). The total sum of n-3 PUFAs (20:5n-3, 22:5n-3, and 22:6n-3) and n-6 PUFAs (18:2n-6, 20:3n-6, 20:4n-6, 22:3n-6, and 22:4n-6), as well as the ratio of n-3 to n-6 fatty acids, was also expressed.

Lymphocyte responses to allergen and mitogen stimulation

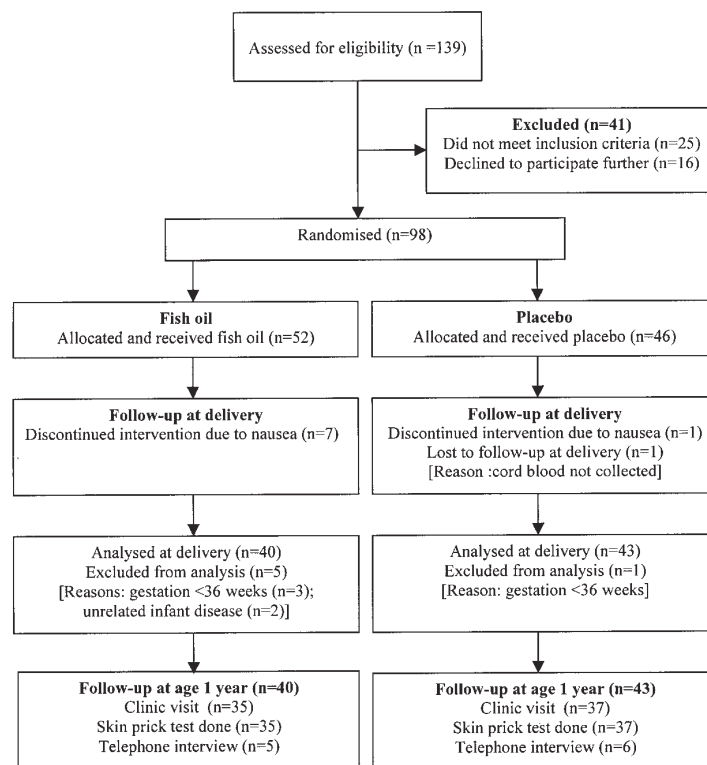
Cord blood samples were collected from the placental vessels into heparinized RPMI (Life Technology, Paisley, United Kingdom) tissue-culture medium. Cord blood mononuclear cells (CBMCs) were isolated from cord blood using Lymphoprep (Nycomed Pharma, Oslo, Norway) gradient centrifugation and cryopreserved for subsequent batch analysis of allergen-specific cytokine responses.⁶ For cytokine analysis, 2×10^9 CBMC/L were cultured in AIM V (Gibco, Life Technology) serum-free medium⁹ for 48 hours with or without allergens, including 10 μ g/mL house dust mite (HDM) extract (CSL, Parkville, Australia), 100 μ g/mL ovalbumin (OVA; Sigma, Castle Hill, Australia), 30 μ g/mL cat hair extract (ALK-Abello, Hølsbøl, Denmark), or 1 μ g/mL PHA mitogen (HA16, Murex, Biotech Ltd, Dartford, United Kingdom). For PHA stimulation (1 μ g/mL), 10^9 CBMC/L were used. Lymphoproliferation responses (after 7 days in culture) were determined by DNA incorporation of tritiated thymidine. Disintegrations per minute were measured on a β -counter (Packard Matrix 9600, Conn). The median of each set of 5 repeats minus the unstimulated control was used as a measure of T-cell proliferation. Detection of a positive response was defined as both a $\Delta > 1000$ dpm plus a stimulation index (ratio of test to unstimulated control) of >2 .

Supernatants were analyzed for IL-5 using an in-house ELISA technique, as previously described.¹⁰ The limit of detection was 3 pg/mL. IL-10, IL-13, and IFN- γ were quantified by an in-house ELISA using a time-resolved fluorometry detection system (Delphia, Perkin-Elmer, Life Sciences, Mass). For detection of IL-10, rat IgG₁ anti-human IL-10 monoclonal antibody (clone JES3-9D7, Pharmingen) was used for capture, and biotinylated rat IgG_{2a} anti-IL-10 monoclonal antibody (clone JES3-12G8, Pharmingen) was used for detection. For detection of IL-13, rat IgG₁ anti-human IL-13 monoclonal antibody (clone JES10-5A2, Pharmingen) was used for capture and biotinylated mouse IgG₁ anti-IL-13 monoclonal antibody (clone B69-2, Pharmingen), for detection. For detection of IFN- γ , mouse IgG₁ anti-human IFN- γ monoclonal antibody (clone NIB42, Pharmingen) was used for capture and biotinylated mouse IgG₁ anti-IFN- γ monoclonal antibody (clone 4S.B3; Pharmingen), for detection. The biotinylated antibody was detected using europium-labeled streptavidin, and fluorescence was quantified using a fluorometer (WALLAC VICTOR,² Perkin-Elmer, Life Sciences). Standard curves, generated using serial dilutions of recombinant human IL-10, IL-13, or IFN- γ (Pharmingen), were linear between 3 and 30,000 pg/mL. Cytokine data were expressed as the difference between the stimulated culture and the control and as detected (>3 pg/mL) or not detected.

Clinical outcomes

Although clinical outcomes were not a prime objective of the study, infants were clinically evaluated at 12 months of age as a secondary outcome measure. This included a detailed history and examination by the same pediatrician (SLP) who remained blinded to the intervention. The main outcome measures were incidence of asthma, atopic eczema dermatitis syndrome, and food allergy. The diagnosis of atopic eczema dermatitis syndrome¹¹ was made in infants with typical skin lesions¹² or physician-diagnosed eczema responsive to topical steroids. The severity of atopic eczema dermatitis syndrome was scored according to the modified SCORAD index,¹³ including the extent of the skin area and severity criteria (erythema, edema, papulation, oozing/crusting, lichenification, and dryness) but with no subjective data (degree of pruritis and sleep loss). The diagnosis of asthma was made in children with recurrent wheezing (3 or more episodes, at least 1 confirmed by pediatrician or general practitioner). All children diagnosed with asthma were

TABLE I. Flow chart showing progress of participants through the trial



responsive to bronchodilator therapy. Chronic cough (in the absence of apparent infection) and recurrent wheeze (on >2 occasions) were also assessed in the clinical history. A skin prick test was performed using a standardized technique¹⁴ and allergen extracts (egg, milk, peanut, house dust mite, cat; Hollister-Stier Laboratories), as well as histamine as a positive control and glycerin as a negative control. A wheal diameter of ≥ 2 mm was considered positive.

Analysis

Only healthy infants (born at ≥ 36 weeks' gestation) were included in the data analysis (to avoid the confounding effects of prematurity). Cytokine and lymphoproliferation data were analyzed as 1) continuous data, described by the geometric mean and 95% CIs, and 2) dichotomous data (detected or not detected). Continuous cytokine data were logarithmically (naturally) transformed to obtain a log-normal distribution, determined by the Kolmogorov-Smirnov test and probability plots, and differences between the fish oil group and the control group were assessed by independent *t* test. Fatty acid levels were expressed as mean and SD of the percentage of total fatty acids, and differences between the groups were assessed by independent *t* test. Differences between the groups for dichotomous data were determined by χ^2 or logistic regression. Associations between fatty acid levels and continuous log-transformed cytokine data were determined by linear regression, expressed as regression coefficient and 95% CI, and adjusted for potential confounding factors that were identified by Pearson correlation. Logistic regression was used to determine the odds ratio (OR) of developing each clinical outcome in the fish oil group compared with the control. All statistical analyses were performed using SPSS software (version 10 for Macintosh). A value of $P < .05$ was considered statistically significant for all analyses.

RESULTS

Characteristics of maternal and neonatal populations

As shown in the trial profile (Table I), 98 women entered the study. There was no significant difference in age, prepregnancy body mass index, allergic status (asthma or allergic rhinitis), or parity between the women in the fish oil and control groups. Eight women discontinued the study because of nausea that they associated with the capsules (7 of these were in the fish oil group). Four infants were subsequently excluded from the data analysis because they were born prematurely (1 in the control group and 3 in the fish oil group); however, there were no differences in gestation between the groups. The reasons for preterm delivery were preeclampsia, complications of placenta previa, and other unknown causes. Specifically, there were no significant differences in birth weight, head circumference, Apgar scores, or the rate of postnatal infections between the groups. Two infants in the fish oil group were excluded for apparently unrelated conditions (1 with a neoplastic condition in later infancy and 1 with an intractable seizure disorder).

Of the 98 women recruited to the study, 83 mothers and their healthy, full-term babies completed the study; 40 in the fish oil group and 43 in the control group.

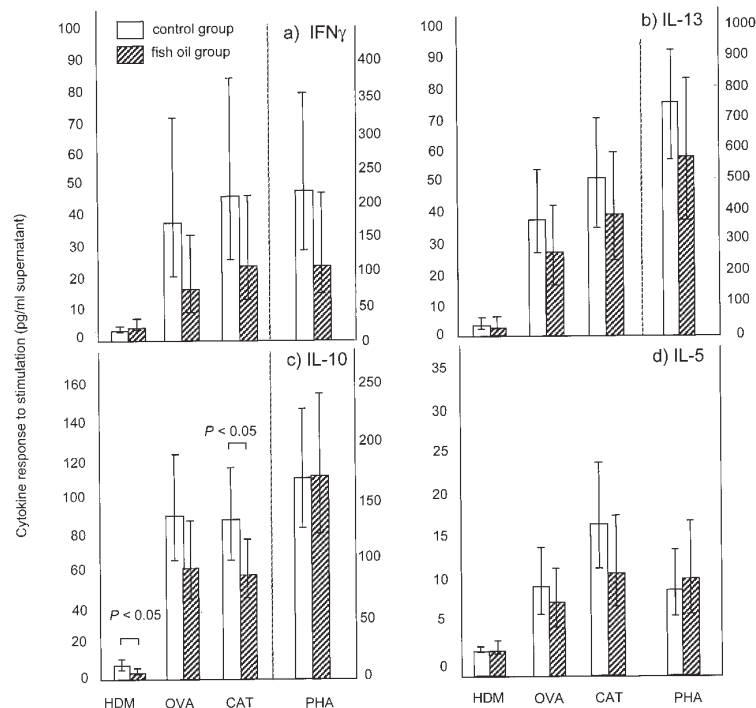


FIG 1. Cytokine response of CBMCs to HDM, OVA, cat, and PHA in the fish oil group (shaded) compared with the placebo group (unshaded). Cytokines are expressed as geometric mean $\pm$ 95% CI (pg/mL). Differences between the 2 groups were determined by independent *t* test. Significance levels are shown, and *P* < .05 is considered to be a significant difference between the 2 groups.

Effects of maternal fish oil supplementation on neonatal n-3 PUFA levels

Fish oil supplementation resulted in a significantly higher proportion (mean $\pm$ SD percentage of total fatty acids) of total n-3 PUFAs in neonatal erythrocyte membranes ($17.75\% \pm 1.85\%$) compared with the control group ($13.69\% \pm 1.22\%$, *P* < .001). Total neonatal n-6 PUFA composition was significantly lower ($25.21\% \pm 1.82\%$) in the fish oil compared with the control group ($29.50\% \pm 1.35\%$, *P* < .001), but there was no significant difference in oleic acid (n-9 PUFA) composition. Background dietary intake assessed by food frequency questionnaires was not different between the 2 groups at study entry or at 30 weeks' gestation.

Effects of maternal fish oil supplementation on CBMC cytokine and lymphoproliferative responses

At birth, although not statistically significant, babies in the fish oil supplementation group generally had weaker cytokine (IL-5, IL-13, IL-10, and IFN- γ) responses to allergens (HDM, OVA, and cat) and mitogen (PHA) (Fig 1). In particular, neonatal in vitro IL-10 response to cat allergen (mean difference, -0.543 ; 95% CI, -1.076 to -0.010 ; *P* = .046) was significantly lower in the fish oil-supplemented group compared with the control group.

When the cytokine data were expressed as dichotomous data (detected or not detected), IFN- γ responses to OVA (*P* = .009) were detected more frequently (33% positive) in the control group compared with in the fish oil group (19%; Table II). There were no differences in the frequency of detectable IL-5, IL-10, or IL-13 responses between the groups (data not shown).

The detection of a lymphoproliferative response to allergens also tended to be lower in the fish oil group compared with the control group. CBMCs from neonates in the control group were 4 times more likely to have a positive proliferative response to OVA allergen (OR, 4.48; 95% CI, 0.87 to 23.07; *P* = .073) and twice as likely to have a positive response to cat (OR, 2.02; 95% CI, 0.69 to 5.88; *P* = .195) than those from the fish oil group, although this was not statistically significant. There was no statistically significant difference in the magnitude (dpm) of lymphoproliferation in the fish oil compared with the control group (data not shown). Response to HDM was very low (generally <1000 dpm) in this population, and there was no difference between the groups.

Association between fatty acid levels and cytokine responses

When the groups were combined (*n* = 83) and analyzed in a linear-regression model, there was a consistent overall trend for all (IL-5, IL-10, IL-13, and IFN- γ) cytokine responses (to allergens and mitogens) to be

TABLE II. IFN- γ cytokine response of CBMCs to allergens expressed as a dichotomous value (detected vs not detected)

Allergen	Olive oil (n = 43), positive (%)	Fish oil (n = 40), positive (%)	P value
HDM	4 (9.3)	7 (17.9)	.251
OVA	33 (76.7)	19 (48.7)	.009
Cat	34 (79.1)	24 (61.5)	.080
PHA	41 (95.3)	35 (89.7)	.292

TABLE III. Associations (regression coefficients) between neonatal red blood cell membrane (n-3 and n-6) fatty acids and neonatal cytokine responses to HDM, OVA, cat, and PHA in the whole population (N = 83)

Cytokine	Stimulant	Fatty acid	Regression coefficient (95% CI)	Adjusted regression coefficient (95% CI)
IFN- γ	HDM	AA (20:4n-6)	0.05 (−0.08, 0.17)	0.04 (−0.09, 0.17)
		EPA (20:5n-3)	−0.02 (−0.31, 0.27)	0.004 (−0.30, 0.30)
		DHA (22:6n-3)	−0.004 (−0.13, 0.12)	0.01 (−0.12, 0.14)
	OVA	AA (20:4n-6)	0.29 (0.02, 0.55)*	0.28 (0.02, 0.54)*
		EPA (20:5n-3)	−0.56 (−1.17, 0.05)	−0.46 (−1.09, 0.16)
		DHA (22:6n-3)	−0.23 (−0.49, 0.04)	−0.19 (−0.46, 0.08)
	Cat	AA (20:4n-6)	0.27 (0.03, 0.51)*	0.25 (0.01, 0.49)*
		EPA (20:5n-3)	−0.57 (−1.14, −0.010)*	−0.47 (−1.04, 0.10)
		DHA (22:6n-3)	−0.22 (−0.47, 0.02)	−0.18 (−0.43, 0.07)
	PHA	AA (20:4n-6)	0.22 (0.01, 0.43)*	0.21 (0.001, 0.42)*
		EPA (20:5n-3)	−0.75 (−1.22, −0.27)†	−0.63 (−1.10, −0.15)*
		DHA (22:6n-3)	−0.20 (−0.42, 0.01)	−0.15 (−0.36, 0.06)
IL-10	HDM	AA (20:4n-6)	0.16 (0.01, 0.31)*	0.14 (−0.01, 0.28)
		EPA (20:5n-3)	−0.36 (−0.71, −0.02)*	−0.34 (−0.68, 0.002)
		DHA (22:6n-3)	−0.12 (−0.27, 0.03)	−0.1 (−0.25, 0.05)
	OVA	AA (20:4n-6)	0.05 (−0.08, 0.19)	0.05 (−0.08, 0.18)
		EPA (20:5n-3)	−0.25 (−0.55, 0.05)	−0.19 (−0.49, 0.11)
		DHA (22:6n-3)	−0.08 (−0.21, 0.05)	−0.06 (−0.19, 0.08)
	Cat	AA (20:4n-6)	0.05 (−0.07, 0.16)	0.05 (−0.07, 0.16)
		EPA (20:5n-3)	−0.31 (−0.57, −0.06)*	−0.31 (−0.57, −0.05)*
		DHA (22:6n-3)	−0.1 (−0.21, 0.02)	−0.08 (−0.20, 0.03)
	PHA	AA (20:4n-6)	0.01 (−0.11, 0.13)	0.01 (−0.11, 0.14)
		EPA (20:5n-3)	−0.04 (−0.32, 0.25)	0.001 (−0.29, 0.29)
		DHA (22:6n-3)	0.04 (−0.09, 0.16)	0.05 (−0.08, 0.17)

*Indicates significance $P < .05$.†Indicates significance $P < .01$.

positively correlated with n-6 PUFA composition and inversely correlated with n-3 PUFA composition of neonatal erythrocyte membranes, after adjusting for confounding factors. In this population, cytokine responses varied with delivery method, parity, and infant gender. Cytokine responses tended to be higher if the women had experienced labor during delivery (rather than having an elective caesarean) and in primiparous compared with multiparous women. For some reason, male infants tended to have higher IL-13 and IL-5 responses to OVA ($P < .05$ and $P < .001$, respectively) and cat ($P < .01$). The relative amount of arachidonic acid (AA, 20:4n-6) was positively associated with the magnitude of IFN- γ responses to OVA ($P = .038$), cat ($P = .040$), and PHA ($P = .048$) after adjusting for parity group, gender, and delivery method (Table III). In contrast, EPA (20:5n-3) was negatively associated with IFN- γ responses to PHA ($P = .011$).

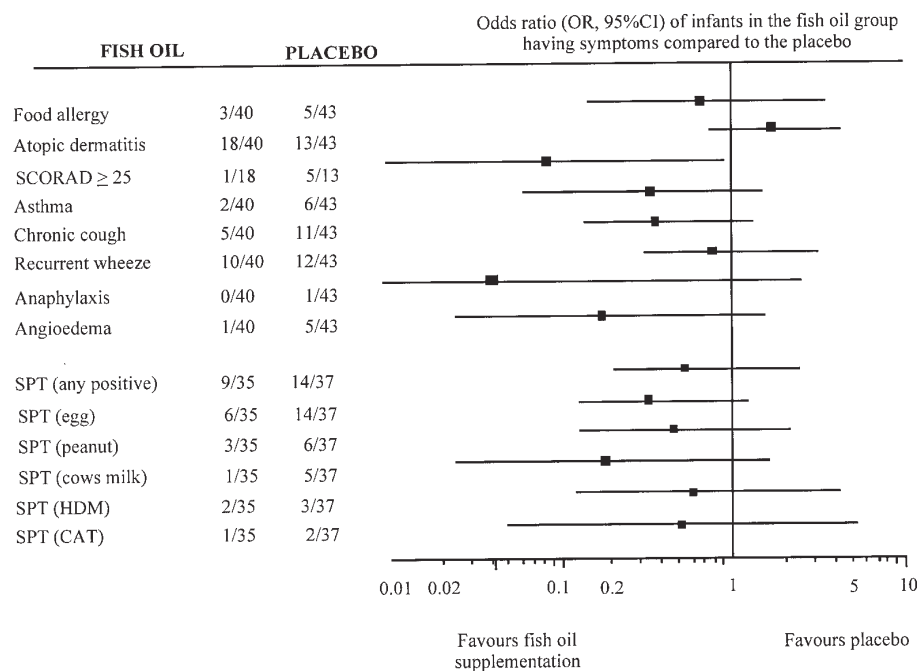
There was a consistent trend for a lack of lymphoproliferation response to allergens and mitogen to be posi-

tively correlated with n-6 PUFA composition and inversely correlated with n-3 PUFA composition of neonatal erythrocyte membranes. In particular, neonates with higher levels of total n-3 PUFAs or a higher n-3 to n-6 PUFA ratio were less likely to demonstrate a positive lymphoproliferative response to cat allergen than were neonates with lower n-3 PUFA amounts in their erythrocyte membranes (OR, 0.806; 95% CI, 0.651 to 0.998, $P = .048$ and OR, 0.037; 95% CI, 0.001 to 0.974; $P = .048$, respectively).

Clinical outcomes at 1 year of age

Although this study was not designed to assess clinical effects of fish oil supplementation, a number of trends and significant differences were observed in the fish oil group. At 1 year of age, a positive skin prick test (OR, 8.16; 95% CI, 2.26 to 26.09; $P < .001$) was the strongest predictor of allergic disease. Infants in the fish oil group were 3 times less likely to be sensitized to egg allergen (OR, 0.34; 95% CI, 0.11 to 1.02; $P = .055$; Table IV).

TABLE IV. Odds ratios and 95% CIs of infants in the fish oil group being skin prick test–positive or having symptoms of allergic disease at 1 year of age compared to the control group (placebo)



Because 37.8% of infants in the control group and 17.1% in the fish oil group were sensitized to egg, this represents an absolute reduction of 20.7% and in this population, a relative risk reduction of 54.6%. Although the OR of atopic dermatitis was higher in the fish oil group compared with the control group (OR, 1.88; 95% CI, 0.77 to 4.65; $P = .167$), among subjects with atopic dermatitis ($n = 31$), those in the fish oil group were 10 times less likely (OR, 0.09; 95% CI, 0.01 to 0.94; $P = .045$) to have severe disease (modified SCORAD index >25 ; Table IV). In general, children in the fish oil group were consistently less likely to develop clinical features, including recurrent wheeze, persistent cough, diagnosed asthma, food allergy, angioedema, or anaphylaxis, compared with the control group (Table IV), although these differences were not statistically significant.

DISCUSSION

It is now recognized that the increase in allergic disease might be the result of failure of normal immune regulation in early life, rather than simple “ T_H2 skewing” of immune responses.¹⁵ This might explain the observations by a number of groups that allergic children have stronger T_H1 and T_H2 responses to allergens.^{16,17} There is now an urgent need to identify environmental factors that might influence immune regulatory pathways in early life and the propensity for allergic disease. To our knowledge, this is the first study to assess the potential immunomodulatory effects of fish oil supplementation in pregnancy on early developing immune responses in infants predisposed to allergic disease. In previous stud-

ies, dietary fish (n-3 PUFA) intake has been associated with a lower incidence of asthma in children.^{18,19} However, until now, all of the intervention studies examining the benefits of fish oil in allergic disease have been in patients with established allergic disease. The lack of apparent benefit, reviewed by Woods et al in 2001,⁵ suggests that effects are limited once allergic immune responses are established. None have looked at fish oil supplementation in high-risk infants before allergic responses are initiated.

Fish oil supplementation in pregnancy achieved a significant increase in n-3 PUFAs in neonatal cell membranes compared with the control group. This was associated with a general trend for attenuated neonatal immune responses to allergens and reduced expression of allergic disease at 1 year of age. Although the consistency of effects seen in multiple aspects of neonatal cellular function suggests a significant biologic phenomenon, the majority of these trends did not reach statistical significance. Although the number of subjects in the study population was based on extensive immunologic data from previous studies, the limitations of this approach are recognized.

The attenuated immunologic responses we observed in neonates delivered to women supplemented with fish oil are consistent with the extensively documented anti-inflammatory properties of n-3 PUFAs.²⁰ Fish oil supplementation increases cell membrane n-3 PUFA composition and inhibits the synthesis of the inflammatory n-6-PUFA-derived eicosanoids leukotriene B_4 and prostaglandin E_2 , known to promote allergic inflammation and IgE responses.^{21,22} Although the mechanisms are still not well understood, these changes are associated with a number of other

immunologic effects, including attenuated lymphoproliferation and reduced proinflammatory cytokine responses (reviewed in Calder et al²⁰), as seen in the present study. The subtle inhibition of both T_H1 and T_H2 responses in this study suggests that PUFAs might exert effects on regulatory aspects of immune function, rather than through simple effects on T_H1 or T_H2 polarization. New “counterregulatory” models of allergy pathogenesis¹⁵ suggest that dysfunction of IL-10–related regulatory mechanisms might underlie the development of allergy, and we might speculate that the anti-inflammatory effects of PUFAs might be mediated through similar regulatory mechanisms. Our findings that IL-10 responses were also attenuated in the fish oil group do not appear to support this. However, IL-10 is produced by numerous cell populations,¹⁵ many of which were not examined here. This highlights the complexity of immunoregulatory networks and the need for further studies to assess the mechanisms of actions of PUFAs and the significance of the observed effects on IL-10 responses.

This study confirms that maternal fish oil supplementation modifies the balance between cellular n-3 and n-6 PUFAs within the fetus and has the capacity to influence neonatal immune function. It also provides preliminary evidence that fish oil might offer potential clinical benefits in reducing the risk of allergic disease and presents a strong case for future larger studies to confirm this effect and address long-term outcomes.

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