

Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy

J. A. Dunstan*, T. A. Mori†‡, A. Barden†‡, L. J. Beilin†‡, A. L. Taylor*, P. G. Holt§ and S. L. Prescott*

*School of Paediatrics and Child Health and †University Department of Medicine, University of Western Australia and ‡Western Australian Institute for Medical Research and §Division of Cell Biology, Telethon Institute for Child Health Research, Perth, Australia

Summary

Background and objectives The epidemiological association between higher dietary n-3 polyunsaturated fatty acids (PUFA) and lower prevalence of asthma, has led to interest in the role of early dietary modification in allergic disease prevention. In this study we examined the effects of maternal n-3 (PUFA)-rich fish oil supplementation on cord blood (CB) IgE and cytokine levels in neonates at risk of developing allergic disease.

Methods In a randomized double-blind, placebo-controlled trial, 83 atopic pregnant women received either fish oil capsules ($n=40$) containing 3.7 g n-3 PUFA/day or placebo capsules ($n=43$) from 20 weeks gestation until delivery. CB cytokine levels (IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, TNF- α and IFN- γ) and total IgE levels were measured and compared between the two groups. Fatty acid composition of red cell membranes was analysed by gas chromatography and the relationships among PUFA, cytokine and IgE levels were examined.

Results Maternal fish oil supplementation resulted in a significant increase in n-3 PUFA levels ($P<0.001$) in neonatal erythrocyte membranes. Neonates whose mothers had fish oil supplementation had significantly lower plasma IL-13 ($P<0.05$) compared to the control group. There was also a significant inverse relationship between levels of n-3 PUFA in neonatal cell membranes and plasma IL-13. There was no difference in levels of IgE and the other cytokines measured.

Conclusions This study provides preliminary evidence that increasing neonatal n-3 PUFA levels with maternal dietary supplementation can achieve subtle modification of neonatal cytokine levels. Further assessment of immune function and clinical follow-up of these infants will help determine if there are any significant effects on postnatal immune development and expression of allergic disease.

Keywords atopy, cord blood, fish oil, IL-13, LCPUFA, pregnancy

Submitted 2 August 2002; revised 17 October 2002; accepted 28 October 2002

Introduction

The events leading to T helper cell 2 (Th2) allergic immune responses are not known, but changing environmental exposures in early life are implicated in the recent increase in allergic diseases [1]. Although not clearly identified, some of these factors may operate before birth to favour persistent Th2 immunity.

Maternal diet in pregnancy represents an important exposure with significant potential to modify immune function and predisposition to allergic disease. One of the most significant recent dietary changes has been decreased consumption of anti-inflammatory omega 3 polyunsaturated fatty acids (n-3 PUFA), with corresponding increased intake of proinflammatory n-6 (omega 6) PUFA. In modern Western diets, the ratio of n-6 to n-3 fatty acids ranges from approximately 20–30:1

instead of the traditional range of 1–2:1 [2]. These changes are reflected in parallel changes in the fatty acid composition of breast milk over the last 20 years [3]. This, coupled with inadequate supplementation of milk formulae, has led to a falling intake of anti-inflammatory n-3 PUFA in early life during the critical period of immune maturation. There is preliminary evidence that lower n-3 PUFA levels in breast milk are associated with infant atopy [4].

Other epidemiological and experimental data also provide a plausible link between these dietary changes and altered immune development. The contrasting immunological effects of n-6 and n-3 PUFA are well described. Omega-6 PUFA [18:2n-6 linoleic acid (LA) and 20:4n-6 arachidonic acid (AA)] promote inflammation, by enhancing mononuclear proliferation, prostaglandin (PG) production (particularly PGE₂), and 'pro-allergic' Th2 differentiation [5]. In contrast, n-3 fatty acids [including 22:6n-3 docosahexaenoic acid (DHA) and 20:5n-3 eicosapentaenoic acid (EPA)] appear to inhibit allergic immune responses through a number of anti-inflammatory properties, including inhibition of PGE₂ [6], lymphoproliferation [7], proinflammatory cytokine responses [8,9] and

Correspondence: S. L. Prescott, Department of Paediatrics, PO Box D184, Princess Margaret Hospital, Perth, WA 6001, Australia.
E-mail: susanp@icmr.uwa.edu.au

expression of MHC class II antigens [10]. These and other anti-inflammatory effects have been utilized in a number of inflammatory conditions with some success (reviewed in [2]).

It is proposed that the more 'proinflammatory' Western diets favour immunological reactivity and Th2 differentiation during the critical time of early development. While increased dietary n-3 PUFA intake has been associated with lower prevalence of asthma and allergic inflammation [11, 12], the benefits of n-3 PUFA in established allergic disease are less clear [13–17]. However, potential dietary effects are likely to be more significant before immune responses are fully established, and suggest a possible role of early n-3 PUFA supplementation in allergic disease prevention. Therefore, in the current study, atopic women were supplemented with fish oil during pregnancy in order to assess the immunological effects of n-3 PUFA in high risk neonates.

Although there are ongoing studies examining the merits of giving n-3 PUFA-rich fish oil in the early post-natal period [18], to our knowledge this is the first study examining the effects of fish oil supplements in pregnancy on neonatal cytokine levels. Because immune responses are initiated before birth and neonates at high atopic risk already have differences in Th1/Th2 responses at birth [19], we hypothesized any beneficial effects of n-3 PUFA on immune development may be greater in the antenatal period. Here we report the effects of maternal fish oil supplementation on cord blood (CB) cytokine and IgE levels.

Materials and methods

Overview/study design

In a double-blind placebo-controlled longitudinal study, 98 allergic women were randomized to receive either a fish oil supplement or a placebo (olive oil) from 20 weeks of pregnancy to delivery. CB was collected for analysis of fatty acid levels, cytokines and total IgE levels.

Study population

All women were booked for delivery at St John of God Hospital, Subiaco, Western Australia between January 2000 and September 2001. Women were defined as allergic if they had a history of doctor-diagnosed allergic rhinitis and/or asthma and one or more positive skin prick test (SPT) to common allergens (house dust mite, grasses, moulds, cat, dog, feathers and cockroach; Hollister-Stier Laboratories, Spokane, WA, USA). A weal size of ≥ 3 mm above the negative control was considered positive. Women were ineligible for the study if they smoked, had other medical problems, complicated pregnancies, seafood allergy or their normal dietary intake exceeded two meals of fish per week. Participants were subsequently excluded if their delivery was pre-term (less than 36 weeks' gestation). To minimize potential confounding factors, at randomization the groups were stratified by parity (no previous term childbirth vs. one or more), pre-pregnancy body mass index (BMI), age and maternal allergy (allergic rhinitis or asthma). Ethical approval for this study was granted by the Princess Margaret Hospital and the St John of God Hospital (Subiaco) Research and Ethics Committees. All women signed a written consent form.

Fish oil (n-3 PUFA) supplements

The supplement group received four (1 g) fish oil capsules per day, comprising a total of 3.7 g of n-3 PUFA with 56.0% as DHA and 27.7% as EPA (Ocean Nutrition, Halifax, Nova Scotia, Canada; confirmed by gas chromatography). The placebo group received four (1 g) capsules of olive oil per day (containing 66.6% n-9 oleic acid and less than 1% n-3 PUFA; Pan Laboratories, Moorebank, NSW, Australia). Twenty weeks' gestation was selected as the 'starting point' for supplementation because the first T cell responses to allergen are not detectable until at least 22 weeks' gestation [20], and earlier supplementation is logistically more difficult. There were no significant changes in background dietary intake in the two groups, assessed by a semiquantitative food frequency questionnaire [21] at 20 and 30 weeks' gestation.

The choice of olive oil as a placebo

Olive oil was chosen as the most suitable lipid for placebo control. Although immunological effects have been reported with olive oil [22], the amount given (4 g) through supplementation does not significantly alter the average daily intake of oleic acid (around 26 g/day) in Australian diets [23].

Samples

CB samples were collected from the placental vessels by venepuncture immediately after delivery, and placed into heparinized Roswell Park Memorial Institute (RPMI, Life Technology, UK) culture medium for transport at room temperature. Plasma samples were isolated within 1 h of collection and stored at -80°C for IgE and cytokine batch analysis later. The dilution factor of CB in RPMI was recorded, and cytokine and IgE levels adjusted accordingly. Total lipids were extracted from red cell membranes (RBC) with methanol and chloroform. CB was also collected into citrate dextrose (ACD) and K_3EDTA (ethylenediamine-tetraacetic acid) anticoagulated tubes (Becton Dickinson, San Jose, CA, USA) for lymphocyte immunophenotyping and total white cell counts.

Measurement of RBC fatty acid levels

RBC membrane incorporation of n-3 and n-6 fatty acids was measured because this gives a long-term indication of dietary levels over the preceding weeks and months (reflecting the half-life of these cells). Fatty acid analyses were carried out as previously described [24]. Briefly, the phospholipid fraction was obtained from total lipid extracts by thin-layer chromatography and the fatty acid methyl esters were analysed by gas liquid chromatography using a Hewlett-Packard model 5980 A gas chromatograph (Hewlett-Packard, Gaithersburg, MD, USA). The fatty acids were expressed as a percentage of the weight of the total fatty acids measured.

CB cytokine levels

CB plasma levels of IL-13, IL-12, TNF- α , IL-5 and IL-4 were measured by enzyme-linked immunosorbent assay (ELISA) as previously described [25]. IL-10, IL-6 and IFN- γ were quantified using time resolved fluorometry (TRF; DELPHIA, Perkin-Elmer Life Sciences, Boston, MA, USA). Briefly, the ELISA method was followed using paired antibodies (Pharmingen, Sydney, NSW, Australia) and the biotinylated antibody was detected using Europium-labelled streptavidin. Fluorescence

dissociated by the addition of low pH enhancement buffer was quantified using a fluorometer (WALLAC VICTOR², Perkin-Elmer Life Sciences). The detection limit was 3 pg/mL for IL-4, IL-5, IL-6, IL-10, IL-12, IL-13 and IFN- γ , and 6 pg/mL for TNF- α . Three plasma samples diluted more than 30% in RPMI at collection were not tested.

CB IgE measurements

IgE measurements were performed on CB plasma by the diagnostic Immunology laboratory at Princess Margaret Hospital using a standardized commercial fluoroimmunoassay (Pharmacia ImmunoCAP for total serum IgE). The limit of detection was 0.35 kUA/L.

Flow cytometric analysis of lymphocyte phenotypes

Whole-blood samples were stained using a two-tube, four-colour direct immunofluorescence reagent kit [Becton Dickinson Immunocytometry Systems (BDIS) MULTitestTM IMK Kit]. Tube A contained 10 μ L anti-CD3 fluorescein isothiocyanate (FITC), anti-CD8 phycoerythrin (PE), anti-CD45 peridinin chlorophyll protein (PerCP) and anti-CD4 allophycocyanin (APC); tube B contained 10 μ L FITC-labelled anti-CD3, PE-labelled anti-CD16, anti-CD56, PerCP labelled anti-CD45 and APC-labelled anti-CD19. Contaminating RBCs were lysed with fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson). Samples were analysed on a flow cytometer (FACSCalibur) and data was acquired and analysed using MultiSET and CELLQuest software (Becton Dickinson). An average of 5000 events falling within the lymphocyte gate were counted in each tube in duplicate. CaliBRITE 3 and APC beads (Becton Dickinson) were employed for instrument calibration and the same voltage settings used for each sample analysed. An adult control sample was used as a quality control check of the system and monoclonal antibody staining was evaluated by calculating a lymphosum [T cells% + natural killer (NK) cells% + B cells%] and monitoring proximity to 100%. Absolute counts of lymphocyte phenotypes were determined from total white blood cell counts (improved Neubauer haemocytometer, Weber Scientific, West Sussex, UK) and a 500-cell white cell differential was performed on a blood smear stained by modified Wrights stain on a automated slide stainer (Miles HemaTek, Rankin Biomedical Co., Clarkson, MI, USA). The total white cell count was adjusted for the presence of nucleated RBCs.

Statistical analysis

All statistical analyses were performed using SPSS software (Version 10 for Macintosh). Log natural transformed data were used for the statistical analysis of all cytokines, IgE and some fatty acids (EPA and total n-3 PUFA) as these data were skewed. Fatty acid data are expressed as mean and SD of the percentage of total fatty acids measured. Cytokine data were described by the geometric mean (GM) and 95% confidence intervals (CI). The transformed data were found to have a log normal distribution using the Kolmogorov-Smirnov test and probability plots.

Differences in fatty acid composition between the fish oil group and the placebo group were assessed by independent Student's *t*-tests. Linear regression was used to examine relationships between continuous variables, after adjusting for confounding factors. Potential confounding factors were

determined by Pearson correlation. Logistic regression was used to examine the relationships between the intervention (using 'group' as a binary variable) and immunological outcomes (continuous variables), after accounting for potential confounding factors. A *P*-value < 0.05 was considered statistically significant for all analyses.

Results

Characteristics of the maternal and neonatal populations

Ninety-eight women were initially recruited to the study. Seven women in the fish oil group and one in the placebo group withdrew from the study because of nausea they attributed to the capsules. Three infants in the fish oil group and one in the control group born before 36 weeks' gestation were excluded from the analysis. Two infants with significant disease were excluded from the analysis. One required chemotherapy for a malignant condition (multisystem Langerhan's histiocytosis) and the other died of a degenerative neurological condition (with progressive leukodystrophy) at several months of age. Although both were in the intervention group, neither condition was linked in any apparent way to the dietary supplementation. CB was inadvertently not collected at one delivery. Characteristics of the 83 mothers and their healthy full-term babies who completed the study (40 in the fish oil group and 43 in the placebo group) are shown (Table 1). There were no significant differences in maternal atopic status, parity, method of delivery or infant gender between the groups. There were no differences in maternal age, or pre-pregnancy BMI or in infant birth weight, birth length, head circumference, gestational age or Apgar scores in the fish oil-supplemented group compared to the placebo group (not shown).

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

At baseline there were no significant differences in the red cell n-6 or n-3 fatty acids between the two groups. Following supplementation, there were significantly higher total n-3 PUFA levels and specifically EPA (mean $\pm$ SD $1.33 \pm 0.52\%$, *P* < 0.001) and DHA (mean $\pm$ SD $10.21 \pm 1.07\%$, *P* < 0.001)

Table 1. Characteristics of the study population

Characteristics	Olive oil (<i>n</i> = 43) Count (%)	Fish oil (<i>n</i> = 40) Count (%)	<i>P</i> -value
Maternal allergy			
Asthma	17 (39.5)	17 (42.5)	0.784
Allergic rhinitis	26 (60.5)	23 (57.5)	
Parity			
Nil	21 (48.8)	18 (45.0)	0.726
One more	22 (51.2)	22 (55.0)	
Delivery method			
Elective caesarean	8 (18.6)	11 (27.5)	0.335
Delivery after labour	35 (81.4)	29 (72.5)	
Gender of the neonate			
Male	22 (51.2)	17 (42.5)	0.429
Female	21 (48.8)	23 (57.5)	

P-values are as shown (significance level *P* < 0.05).

levels in the fish oil group compared to the placebo group (EPA mean $\pm$ SD $0.37 \pm 0.28\%$; DHA mean $\pm$ SD $7.30 \pm 0.95\%$; Fig. 1). Levels of n-6 PUFA AA were significantly lower in the fish oil group (mean $\pm$ SD 15.02 ± 1.44 , $P < 0.001$), compared with the placebo group (mean $\pm$ SD $17.45 \pm 1.17\%$) with a resulting increase in the ratio of n-3/n-6 PUFA levels in fetal cell membranes compared to the placebo group. There was no difference in the levels of oleic acid (18:1n-9) between the groups, indicating that the olive oil placebo did not have any effect on total dietary intakes of oleic acid.

Effects of fish oil supplementation on CB cytokine and IgE levels

IL-13 levels were significantly lower (GM 9.61, 95% CI 5.46–16.93; $P = 0.025$) in neonates whose mothers received fish oil supplements in pregnancy compared to the placebo group (GM 26.32, 95% CI 13.44–51.55; Fig. 2). Cord plasma IL-13 was detectable in 64% ($n = 27$) of plasma samples from the placebo group compared to 44.7% ($n = 17$) of samples from the fish oil group ($P = 0.08$). Neonates delivered by elective caesarean were less likely [odds ratio (OR) 0.16, 95% CI 0.05–0.54; $P = 0.003$] to have detectable cord plasma IL-13 and had lower IL-13 levels (GM 6.39, 95% CI 2.59–15.78; $P = 0.02$) compared to those whose mothers went into active labour (GM 21.41, 95% CI 12.88–35.59). Female infants were more likely (OR 2.75, 95% CI 1.11–6.82; $P = 0.03$) to have IL-13 detected in the CB plasma, and had higher levels (GM 25.87, 95% CI 13.79–48.53; $P = 0.03$) compared to male infants (GM 9.80, 95% CI 5.23–18.35). Because gender of the infant and method of delivery did not differ significantly between the groups, they were unlikely to contribute to the difference in IL-13 between the two groups. This was confirmed with multivariate linear regression (for continuous data) or logistic regression (for categorical data). There were no relationships between IL-13 and neonatal growth parameters.

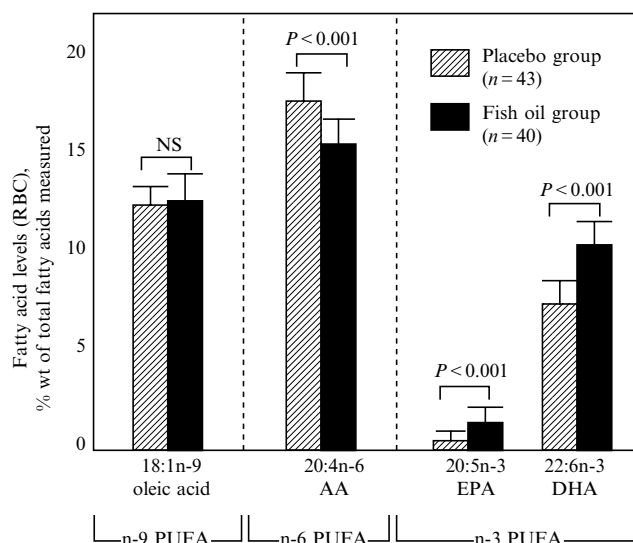


Fig. 1. Comparison of neonatal red cell membrane fatty acid composition in the fish oil- and placebo-supplemented groups. This illustrates the effects of maternal fish oil supplementation on neonatal erythrocyte PUFA levels. Differences between the fish oil group (dark bars) and the placebo group (light bars) were assessed by independent Student's *t*-test (significance values are as shown). Fatty acid levels are expressed as mean and standard deviation of percentage of total fatty acids measured.

There were no differences in IFN- γ levels between the groups; however, this cytokine was below detection in most (more than 95%) of the cord plasma samples. Most other cytokines (TNF- α , IL-4, IL-5, IL-6, IL-10, IL-12) were also only detectable in a minority of cases (less than 10% of neonates) and there were no differences in the magnitude or the frequency of detection between the groups (not shown).

Total IgE levels in plasma were only detectable (> 0.35 kU/L) in 5% ($n = 4$) of this neonatal population, and there were no significant differences between the fish oil group and the placebo group (not shown). There was no relationship between IL-13 and IgE levels.

Effects of fish oil supplementation on lymphocyte subsets in CB

There was no significant difference in the frequency of lymphocyte subsets (% and absolute concentration) between the two groups, with respect to total T cells (CD3 $^{+}$), T helper cells (CD4 $^{+}$), T suppressor cells (CD8 $^{+}$), NK cells (CD16 $^{+}$ and CD56 $^{+}$) and B cells (CD19 $^{+}$) (not shown).

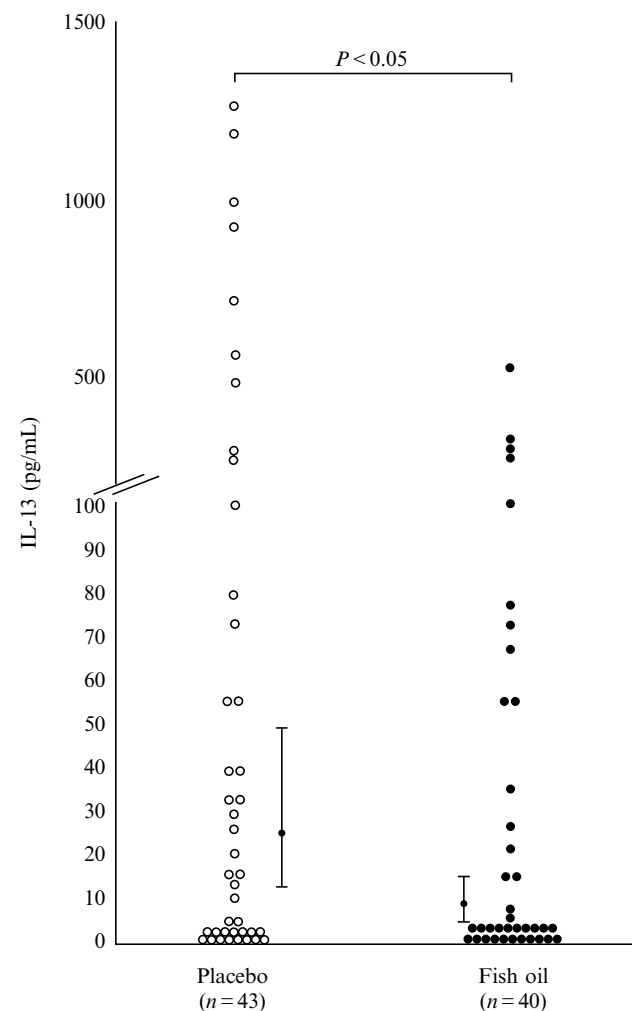


Fig. 2. Comparison of free neonatal IL-13 in the fish oil- and placebo-supplemented groups. This illustrates the individual IL-13 levels in each group with GM IL-13 (pg/mL) $\pm$ 95% CI. Differences between the fish oil group (dark circles) and the placebo group (light circles) were assessed by independent Student's *t*-test (significance values are as shown).

Relationship between neonatal n-3 PUFA levels and CB cytokine levels

The fatty acid data from both groups were combined to achieve a range of DHA levels (5.17–12.77% of the total fatty acids measured) to determine any associations between DHA status and cytokine (IL-13) levels. There was a significant inverse relationship between 22:6n-3 (DHA) and plasma IL-13 after accounting for potential confounding effects (parity, gender and delivery method; $P = 0.04$; Table 2). The range for 20:5n-3 (EPA) was much smaller (0.18–2.82% of total fatty acids) and, although the same trend was seen, this was not statistically significant. In contrast, n-6 PUFA levels tended to be positively correlated to circulating IL-13 in cord plasma, although this was not statistically significant. There were no other significant relationships between PUFA levels and cytokine or IgE levels in cord plasma.

Relationship between neonatal plasma IL-13 levels and CB lymphocyte subsets

There was a significant positive relationship between plasma IL-13 levels and NK cells ($CD56^+/CD16^+$ $P = 0.02$) and B cells ($CD19^+$ $P = 0.02$) in CB (Table 3). However, these relationships did not remain statistically significant after controlling for differences in gender, delivery method and parity. There was no relationship between NK or B cell numbers and n-3/n-6 PUFA levels. There were also no relationships between numbers or percentages of CD4 and CD8 T cells and cytokine levels (Table 3) or PUFA levels (not shown).

Discussion

Early studies of immunological changes in pregnancy suggested that 'Th2 skewing' of immune responses at the maternal–fetal interface is important for fetal survival [26]. Although new research suggests more complex immunological changes that are not fully explained by the Th1/Th2 paradigm (reviewed in [27, 28]), this model has been immensely useful. With recognition that abnormalities of early immune programming may contribute to persistent Th2 responses and allergic disease, there is also growing interest in factors that regulate Th1/Th2 responses in the perinatal period. Although multiple environmental changes are likely to be contributing to the increasing propensity for Th2 responses in infancy, to our knowledge this is the first study examining the relationship between maternal dietary PUFA and neonatal Th1/Th2 cytokine levels.

In addition to epidemiological associations with allergic disease, the well-recognized anti-inflammatory properties of n-3 PUFA have provided a plausible pathway of influence during early immune development. Some of the anti-inflammatory properties of n-3 PUFA are mediated through competitive inhibition of PGE_2 synthesis. Unlike n-6 PUFA, which are preferentially metabolized to produce PGE_2 and other inflammatory mediators [29], n-3 PUFA derived metabolites [leukotriene (LT) B_5 and PGE_3] are less inflammatory. In addition to its inflammatory properties, PGE_2 also inhibits Th1 IFN- γ responses [30, 31] and favours Th2 differentiation (reviewed in [5]). Neonatal lymphocytes are known to be more sensitive to these 'Th1 inhibitory' effects of PGE_2 [32], and early studies

Table 2. Relationship (regression coefficients) between neonatal red cell membrane fatty acid levels and cord plasma IL-13 levels*

Polyunsaturated fatty acid	Regression coefficient (95% CI)	Adjusted regression coefficient† (95% CI)
n-6 PUFA		
Linoleic acid (18:2n-6)	− 0.53 (− 1.57, 0.52)	− 0.45 (− 1.46, 0.56)
Dihomo γ -linolenic acid (20:3n-6)	0.72 (− 0.22, 1.66)	0.71 (− 0.21, 1.62)
Arachidonic acid (20:4n-6)	0.07 (− 0.30, 0.94)	0.01 (− 0.23, 0.26)
22:3n-6	0.32 (− 0.30, 0.94)	0.28 (− 0.32, 0.88)
Docosatetraenoic acid (22:4n-6)	0.87 (− 0.15, 1.89)	0.81 (− 0.19, 1.80)
Total n-6‡	0.09 (− 0.08, 0.25)	0.06 (− 0.11, 0.22)
n-3 PUFA		
Eicosapentaenoic acid (20:5n-3)	− 0.40 (− 0.98, 0.18)	− 0.40 (− 0.97, 0.16)
Docosapentaenoic acid (22:5n-3)	− 0.22 (− 0.83, 0.38)	− 0.31 (− 0.90, 0.27)
Docosahexaenoic acid (22:6n-3)	− 0.27 (− 0.52, − 0.02)¶	− 0.25 (− 0.49, − 0.01)¶
Total n-3§	− 2.73 (− 5.45, − 0.01)¶	− 2.70 (− 5.35, − 0.05)¶

*Results of simple and multiple regression analyses with free cord plasma IL-13 and neonatal red blood cell n-3 and n-6 PUFA. †Adjusted for gender, parity and method of delivery. ‡Sum 18:2, 20:3, 20:4, 22:3, 22:4. §Sum 20:5, 22:5, 22:6. ¶Indicates significance $P < 0.05$.

Table 3. Relationship (regression coefficients) between neonatal lymphocyte subsets and cord plasma IL-13 levels*

Cell marker	Regression coefficient (95% CI)	Adjusted regression coefficient† (95% CI)
CD3 ⁺ (T cells)	0.41 (− 0.08, 0.89)	0.34 (− 0.13, 0.81)
CD4 ⁺ (T helper cells)	0.62 (− 0.02, 1.26)	0.53 (− 0.10, 1.17)
CD8 ⁺ (T suppressor cells)	0.25 (− 1.09, 1.60)	0.29 (− 0.99, 1.57)
CD19 ⁺ (B cells)	1.54 (0.31, 2.77)‡	1.05 (− 0.19, 2.29)
CD16 ⁺ 56 ⁺ (NK cells)	0.85 (0.15, 1.55)‡	0.54 (− 0.19, 1.28)

*Results of simple and multiple regression analyses with free cord plasma IL-13 and neonatal lymphocyte subsets. †Adjusted for gender, parity and method of delivery. ‡Indicates significance $P < 0.05$.

suggested that the normally impaired IFN- γ Th1 responses in the neonatal period can be reversed by inhibiting PGE₂ with indomethacin [32]. This suggests that subtle changes in fatty acid and PG metabolism could alter the Th1/Th2 balance in the neonatal period. Furthermore, diets rich in n-6 PUFA may be directly implicated in the impaired neonatal Th1 responses that have been associated with allergic disease ([19, 33–36] and others). A number of studies have described altered fatty acid levels in allergic mothers and their infants ([37, 38] and others), but further studies are required.

In this study, we observed that maternal dietary supplementation with n-3 PUFA was associated with significantly lower IL-13 levels in cord plasma. There were also consistent correlations between IL-13 levels and n-3 PUFA levels, in particular DHA. This finding supports a relationship between maternal dietary PUFA and neonatal immune function. The origin of the IL-13 is not clear, but is more likely to be derived from the fetus than the placenta late in pregnancy [39, 40]. The predictive value of neonatal Th2 responses, including IL-13, and risk of allergic disease is not clear. Some studies have higher Th2 responses in association with atopic risk [41, 42], others have observed lower levels [39, 43]. The association between high atopic risk and weaker neonatal Th1 responses appears more consistent [32–36], and may play a role in the reduced ability to develop mature Th1 responses in the post-natal period. In the present study, lower IL-13 levels may reflect a subtle shift in the cytokine balance, favouring Th1 immunity, which is not evident by measuring low-level plasma IFN- γ (below detection in most infants). The mechanism of action is also not clear, but could be mediated through changes in neonatal PGE₂ synthesis, which is known to be inhibited by maternal n-3 PUFA supplementation [6]. Omega-3 PUFA have many other immunomodulatory effects (reviewed in [44]), and have been also shown to promote tolerance at the maternal–fetal interface by suppressing MHC Class II expression [10]. It is also possible that immunological effects at the maternal–fetal interface may occur as the secondary result of fatty acid changes on maternal immune responses. Although we have demonstrated a direct relationship between neonatal fatty acid status and neonatal immune function, the effects of this dietary modification on maternal immune response were not assessed in this study.

PUFA may also have effects on NK function and although this is less well documented [44] it appears that high-dose DHA supplementation can decrease NK cell activity [45, 46]. Although fish oil supplementation was not associated with differences in NK cell numbers in the present study, function was not assessed. However, the possible relationship between IL-13 and NK cell numbers in this study suggests a potential effect. The significance of this observation is unclear, but highlights the growing need to define the role of NK cells in gestation and immune development. Although initially believed to be a threat to pregnancy, NK cells are now believed to play an immunoregulatory role at the maternal–fetal interface (reviewed in [27]). Depending on the prevailing cytokine and milieu maturation stage, NK cells may also exist in subsets producing type 1 and type 2 cytokines, including IL-13 [47]. We may speculate that variations in NK phenotype may also have implications for immune maturation and atopic risk.

While these immunological changes appear subtle, they may have a significant effect on developing immune responses, which are plastic and vulnerable to influence. Our findings add

weight to the speculation that dietary factors can influence the immunological processes that are central to the pathogenesis of atopy, and accordingly that changing dietary patterns may be contributing to the increasing prevalence of allergic disease [5, 48]. We have demonstrated preliminary evidence that altered membrane PUFA profiles during gestation may influence immunological function during this critical time of development. Ongoing studies are needed to examine the effects of early n-3 PUFA supplementation on evolving allergen-specific responses and the effects on subsequent allergic disease. These issues will be subsequently addressed in this population.

Acknowledgements

We wish to acknowledge the staff and patients who assisted in this study. We are particularly grateful to the obstetricians and midwives at St John of God Hospital, Subiaco, Western Australia. We thank Professor Scott Weiss, Harvard Medical School for his role in the planning and design of this study. We also wish to thank Ms Lynette McCahon for technical assistance, Ms Elaine Pascoe for statistical advice and Dr Thierry Venaille for his advice and support during the study design. The study was supported by grants from the NH & MRC and Raine Medical Research Foundation, Australia.

References

- Holt P. Post-natal maturation of immune competence in infancy and childhood. *Pediatr Allergy Immunol* 1995; 6:59–70.
- Simopoulos AP. Essential fatty acids in health and chronic disease. *Am J Clin Nutr* 1999; 70:560S–69S.
- Makrides M, Simmer K, Neumann M, Gibson R. Changes in the polyunsaturated fatty acids of breast milk from mothers of full-term infants over 30 weeks of lactation. *Am J Clin Nutr* 1995; 61:1231–3.
- Duchen K, Yu G, Bjorksten B. Atopic sensitization during the first year of life in relation to long-chain polyunsaturated fatty acid levels in human milk. *Pediatr Res* 1998; 44:478–84.
- Black P, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J* 1997; 10:6–12.
- Sorensen JD, Olsen SF, Pedersen AK, Boris J, Secher NJ, FitzGerald GA. Effects of fish oil supplementation in the third trimester of pregnancy on prostacyclin and thromboxane production. *Am J Obstet Gynecol* 1993; 168:915–22.
- Soyland E, Nenseter MS, Braathen L, Drevon CA. Very-long-chain n-3 and n-6 polyunsaturated fatty acids inhibit proliferation of human T-lymphocytes *in vitro*. *Eur J Clin Invest* 1993; 23:112–21.
- Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J Leukoc Biol* 1993; 54:599–603.
- Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor α and interleukin 1 β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr* 1996; 63:116–22.
- Khair-el-Din TA, Sicher SC, Vazquez MA, Wright WJ, Lu CY. Docosahexaenoic acid, a major constituent of fetal serum and fish oil diets, inhibits IFN γ -induced Ia-expression by murine macrophages *in vitro*. *J Immunol* 1995; 154:1296–306.
- Hodge L, Salome C, Peat J, Haby M, Xuan W, Woolcock A. Consumption of oily fish and childhood asthma risk. *Med J Aust* 1996; 164:137–40.

- 12 Peat J, Salome C, Woolcock A. Factors associated with bronchial hyper-responsiveness in Australian adults and children. *Eur Respir J* 1992; 5:921–9.
- 13 Dry J, Vincene D. Interest of fish oil in asthma: results of a 1-year double-blind study. *Int Arch Allergy Appl Immunol* 1991; 95:156–7.
- 14 Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am J Clin Nutr* 1997; 65:1011–7.
- 15 Villani F, Comazzi R, De Maria P, Galimberti M. Effect of dietary supplementation with polyunsaturated fatty acids on bronchial hyper-reactivity in subjects with seasonal asthma. *Respiration* 1998; 65:265–9.
- 16 Arm JP, Horton CE, Mencia-Huerta JM et al. Effect of dietary supplementation with fish oil lipids on mild asthma. *Thorax* 1988; 43:84–92.
- 17 Thien FC, Atkinson BA, Khan A, Mencia-Huerta JM, Lee TH. Effect of dietary fish oil supplementation on the antigen-induced late-phase response in the skin. *J Allergy Clin Immunol* 1992; 89:829–35.
- 18 Mahrshahi S, Peat JK, Webb K et al. The childhood asthma prevention study (CAPS): design and research protocol of a randomized trial for the primary prevention of asthma. *Control Clin Trials* 2001; 22:333–54.
- 19 Prescott SL, Macaubas C, Smallacombe T et al. Reciprocal age-related patterns of allergen-specific T cell immunity in normal versus atopic infants. *Clin Exp Allergy* 1998; 28 (Suppl. 5):39–44; discussion 50–1.
- 20 Jones A, Miles E, Warner J, Colwell B, Bryant T, Warner J. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Ped Allergy Immunol* 1996; 7:109–16.
- 21 CSIRO Division of Human Nutrition. Diet and Health Booklet. Adelaide, SA: CSIRO, Division of Human Nutrition, 1996.
- 22 Yaqoob P, Knapper JA, Webb DH et al. Effect of olive oil on immune function in middle-aged men. *Am J Clin Nutr* 1998; 67:129–35.
- 23 CSIRO Division of Human Nutrition. Australian food and nutrition surveys 1988 & 1993. Adelaide, SA: CSIRO, Division of Human Nutrition, 1996.
- 24 Mori TA, Burke V, Puddey IB et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* 2000; 71:1085–94.
- 25 Ng TW, Holt PG, Prescott SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. *Allergy* 2002; 57:207–14.
- 26 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993; 14:353–6.
- 27 Zourbas S, Dubanchet S, Martal J, Chaouat G. Localization of proinflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines at the foeto–maternal interface during murine pregnancy. *Clin Exp Immunol* 2001; 126:519–28.
- 28 Chaouat G, Zourbas S, Ostojic S et al. A brief review of recent data on some cytokine expressions at the materno–foetal interface which might challenge the classical Th1/Th2 dichotomy. *J Reprod Immunol* 2002; 53:241–56.
- 29 Sprecher H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim Biophys Acta* 2000; 1486:219–31.
- 30 Snijderwint F, Kalinski P, Wieringa E, Bos J, Kapsenberg M. Prostaglandin E₂ differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* 1993; 150:5321–9.
- 31 Gold K, Weyand C, Goronzy J. Modulation of helper T cell function by prostaglandins. *Arthritis Rheum* 1994; 37:925–33.
- 32 Wakasugi N, Virelizier J. Defective interferon- γ production in the human neonate I dysregulation rather than intrinsic abnormality. *J Immunol* 1985; 134:167–76.
- 33 Rinas U, Horneff G, Wahn V. Interferon gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE levels. *Ped Allergy Immunol* 1993; 4:60–4.
- 34 Martinez F, Stern D, Wright A, Holberg C, Taussig L, Halonen M. Association of interleukin-2 and interferon- γ production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995; 96:652–60.
- 35 Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon- γ production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24:423–30.
- 36 Tang MLK, Kemp AS, Thorburn J, Hill D. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994; 344:983–5.
- 37 Galli E, Picardo M, Chini L et al. Analysis of polyunsaturated fatty acids in newborn sera: a screening tool for atopic disease? *Br J Dermatol* 1994; 130:752–6.
- 38 Yu G, Bjorksten B. Serum levels of phospholipid fatty acids in mothers and their babies in relation to allergic disease. *Eur J Pediatr* 1998; 157:298–303.
- 39 Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. *J Allergy Clin Immunol* 2000; 105:951–9.
- 40 Dealtry GB, Clark DE, Sharkey A, Charnock-Jones DS, Smith SK. Expression and localization of the Th2-type cytokine interleukin-13 and its receptor in the placenta during human pregnancy. *Am J Reprod Immunol* 1998; 40:283–90.
- 41 Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN- γ -producing cells in *in vitro* activated cord blood mononuclear cells. *Clin Exp Immunol* 2001; 126:390–6.
- 42 Liao S, Liao T, Chiang B et al. Decreased production of IFN γ and decreased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin Exp Allergy* 1996; 26:397–405.
- 43 Prescott S, Macaubas C, Holt B et al. Transplacental priming of the human immune system to environmental allergens. universal skewing of initial T cell responses towards Th-2 cytokine profile. *J Immunol* 1998; 160:4730–7.
- 44 Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* 2001; 36:1007–24.
- 45 Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *J Nutr* 2001; 131:1918–27.
- 46 Kelley DS, Taylor PC, Nelson GJ et al. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids* 1999; 34:317–24.
- 47 Loza MJ, Peters SP, Zangrilli JG, Perussia B. Distinction between IL-13+ and IFN- γ + natural killer cells and regulation of their pool size by IL-4. *Eur J Immunol* 2002; 32:413–23.
- 48 Weiss S. Diet as a risk factor for asthma. In: CIBA Foundation Symposium 1997. New York: Wiley, 1997.