

EDITORIAL

Polyunsaturated fatty acids and cytokine profiles: a clue to the changing prevalence of atopy?

There has been an increase in the prevalence of atopic disease in the last four decades in a number of populations [1–3]. Such an increase must be due to alterations in environmental factors. Atopic disease is characterized by a bias in favour of Th2-type cytokine production [4], and thus factors that favour this bias may predispose individuals to develop atopic responses. Since sensitization to allergens may occur early in life [5], factors relating to early life exposure may be involved.

The time-scale of the increased prevalence of atopic disease is mapped by large changes in the type of fat consumed in those same populations: over the last four decades there has been a decrease in the intake of saturated fats and an increase in the intake of vegetable oil-based products, rich in the n-6 family of polyunsaturated fats [6]. The major n-6 polyunsaturated fatty acid consumed in the human diet is linoleic acid, and this fatty acid is converted to arachidonic acid (Fig. 1). Human inflammatory and immune cells contain high proportions of arachidonic acid [7, 8] and its principal functional role is as a substrate for the synthesis of the eicosanoid family of mediators [9]. Metabolism of arachidonic acid by cyclooxygenase gives rise to the 2-series prostaglandins (PG) such as PGE₂ (Fig. 1). The pattern of change in dietary fatty acid intake over the last 40 years has been suggested to be responsible for the increase in atopic disease [10–12]. This is because PGE₂ synthesized from arachidonic acid exerts effects that could promote the development of the Th2-type phenotype that underlies atopic disease. PGE₂ has been reported to increase the production of IL-4 [13], IL-5 [14–16] and IL-10 [17] by human T cells. Furthermore, PGE₂ potentially decreases the production of Th1-type cytokines such as IFN- γ and IL-2 [14–16], which would be expected to relieve the Th1-mediated inhibition of development of the Th2 phenotype [18]. Thus, it appears that PGE₂ suppresses Th1 cytokine production, so favouring a Th2 bias. A recent study indicates that significant amounts of PGE₂ are present in human amniotic fluid at the time at which atopic sensitization might occur [19].

There is a second major class of polyunsaturated fatty acids, the n-3 fatty acids. As the consumption of vegetable oil-based products has increased, the consumption of n-3 polyunsaturated fatty acids has decreased in both absolute and relative (to the n-6 class) terms [20]. Oily fish are naturally rich in the long-chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are also found in the commercial preparations known as 'fish oils'. When fish oil (or oily fish) is consumed, the amount of arachidonic acid in human inflammatory and immune cells is decreased [7, 21–23]. In studies of healthy adults this has been shown to result in a decreased capacity for production of arachidonic acid-derived eicosanoids such as PGE₂ [22–24]. Since increased consumption of fish oil results in decreased capacity to produce PGE₂, it has been suggested that the intake of n-3

fatty acids from oily fish and fish oil be increased as a strategy to prevent atopic disease [10–12].

Biochemical measurements suggest an inverse relationship between n-3 fatty acid status and atopic disease. The proportions of EPA, docosapentaenoic acid and DHA were higher in umbilical cord serum phospholipids from non-allergic compared with allergic mothers [25]. Higher n-3 polyunsaturated fatty acids in breast milk were associated with decreased likelihood of atopy in the infants [26]. The proportions of DHA and of total n-3 fatty acids were higher in the serum phospholipids from 12 to 15-year-old non-atopic controls than in those from children with asthma and/or atopic dermatitis [27].

There is also epidemiological evidence to support a protective role of long-chain n-3 fatty acids in atopic disease. Data from the 1st and 2nd National Health and Nutrition Surveys in the USA found that dietary fish intake was positively associated with FEV₁ [28] and protected against wheezing [29], respectively. Australian schoolchildren who included oily fish in their diet had a lower likelihood of having asthma (odds ratio 0.26) than children who did not consume oily fish [30]. Schoolchildren who went on to develop atopy had previously consumed more margarine and less butter (and so more n-6 fatty acids) than those who did not develop atopy [31].

Despite a biologically plausible mechanism and the supportive biochemical and epidemiological data, the key to demonstrating a protective effect of increased long-chain n-3 fatty acid consumption towards atopic disease must come from well-designed, placebo-controlled intervention studies. Given that atopic sensitization may occur *in utero* [5], such studies need to be performed in pregnant women. Studies conducted in other settings have established that increased consumption of long chain n-3 fatty acids in pregnancy results in increased amounts of these fatty acids in maternal plasma and erythrocytes [32, 33], in cord blood plasma [33], and in umbilical veins and arteries [33, 34]. However, these studies have largely focused on the potential beneficial effect of DHA on the mental and visual development of the newborn, rather than on immunological development and there has been rather little attention paid to the latter. In this issue of *Clinical and Experimental Allergy*, there is a report on the effect of maternal fish oil consumption during pregnancy on cord blood cytokine concentrations [35]. The mothers involved were proven to be allergic through clinical history and positive skin prick test results. They consumed fish oil providing 1.1 g EPA + 2.2 g DHA per day from 20 weeks gestation until delivery. Typical daily intakes of EPA plus DHA in adult individuals who do not regularly consume oily fish (and do not consume fish oil capsules) are < 0.25 g [20]. One serving of oily fish such as salmon, trout, pilchards, sardines, mackerel or kippers will provide between 1.5 and 3.5 g EPA + DHA [20]. In the study [35] fish oil was provided as four capsules (each 1 g) daily. The placebo was olive oil provided in the same manner.

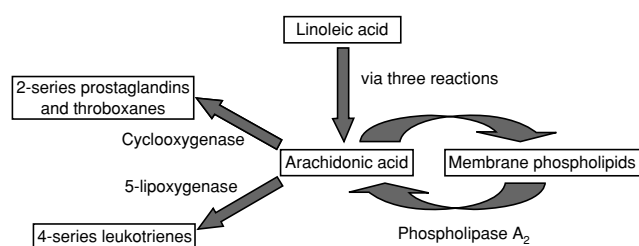


Fig. 1. An outline of n-6 polyunsaturated fatty acid metabolism. The simplest n-6 fatty acid (linoleic acid) cannot be synthesized *de novo* in mammalian cells and must be obtained from the diet. Once consumed, linoleic acid can be metabolized to arachidonic acid. Arachidonic acid can be mobilized from cell membranes in response to various stimuli. Once released, arachidonic acid can act as a substrate for cyclooxygenase enzymes giving rise to prostaglandins (PG). PGE₂ regulates the production of Th1- and Th2-type cytokines. Arachidonic acid is also a substrate for 5-lipoxygenase, giving rise to 4-series leukotrienes. These are mediators of allergic inflammation.

Ultimately data from about 40 pregnancies in each of the two groups was available. The proportion of arachidonic acid was lower (by 15%) in cord blood erythrocytes in the fish oil group, while the proportions of EPA and DHA were higher (by 260% and 40%, respectively). The ratio of arachidonic acid to EPA was 47 in the placebo group and 11 in the fish oil group. Thus, this study demonstrates that providing long-chain n-3 fatty acids during pregnancy alters the fatty acid composition of cord blood erythrocytes; this is consistent with earlier studies of cord blood plasma [e.g. 33]. It is assumed that this change occurred earlier in development and that it occurred in the membranes of those cells involved in the atopic sensitization process. The changes in fatty acid composition of cord blood erythrocytes are fairly consistent with those reported for blood mononuclear cells from healthy adults who consumed fish oil providing 2.1 g EPA + 1.1 g DHA per day for 12 weeks [21]. The really novel aspects of this new study [35] relate to the assessment of cord blood immune phenotypes and cytokine profiles. There was no effect of fish oil on the numbers or proportions of T cells, helper T cells, suppressor T cells, B cells or natural killer cells in cord blood. This observation is in agreement with studies in adults showing no effect of fish oil on circulating immune cell numbers [21, 36]. IgE and seven cytokines were detectable in only a small minority of cord plasma samples. However, IL-13 was detected in 64% of cord plasmas in the placebo group and 45% in the fish oil group. Overall cord plasma IL-13 concentrations were significantly lower (by 65%) in the fish oil group. This is the first study to report such an effect. There was a significant inverse relationship between the proportion of DHA in cord blood erythrocytes and cord plasma IL-13. The negative relationship with EPA and the positive relationship with linoleic acid and IL-13 concentration were not significant. Nevertheless, these relationships are in accordance with the underlying hypothesis that n-6 fatty acids predispose to, and n-3 fatty acids protect against, atopy. Erythrocytes are, of course, not the source of IL-13 and in relating the composition of erythrocytes to IL-13 concentrations the authors appear to assume that erythrocytes present a fatty acid composition that is representative of that of the cells involved in cytokine production. In general, this is probably true since after chronic administration of fish oil most cells will

be enriched in EPA and DHA and will contain less arachidonic acid. However, the precise quantitative nature of the changes in fatty acid composition may differ among different cell types. Thus, it would have been very useful, and informative, to have presented the fatty acid composition of cord blood lymphocytes (or mononuclear cells). Additionally, the authors have assumed that the fatty acid composition and cytokine differences observed in cord blood represent those that pertained at the much earlier time of sensitization. This may or may not be the case. Given that most of the cytokines investigated were not detectable in most plasma samples, it would have been useful to have studied cord blood lymphocyte responses to *ex vivo* stimulation with allergens. The cytokine profiles produced by cord blood cells in response to allergens have been reported to be predictive of later atopy [37–39]. Finally, given that a decrease in capacity to produce PGE₂ is central to the hypothesis of a protective effect of n-3 fatty acids, it would have been informative to have measured plasma PGE₂ concentrations or to have measured PGE₂ production by allergen-stimulated cord blood cell cultures.

Although IL-13 is regarded as an 'IL-4-like' Th2-type cytokine, its role in predisposing to atopy is unclear. Stimulated blood cells from atopic children produced more IL-13 (and IL-4 and IL-5) than those from non-atopics [40]. Furthermore, purified cord blood T cells from neonates who went on to develop atopy over the first 12 months of life produced more IL-13 than those from neonates who went on to remain non-atopic [41]. These authors suggested that increased IL-13 production by cord blood T cells may be a useful marker of risk for subsequent atopic disease [41]. These observations suggest that the new findings of Dunstan et al. [35] may ultimately represent a real clinical benefit from fish oil intervention in pregnancy. However, other workers reported that cord blood cells from neonates with a parental history of atopy produced less IL-13 than those from neonates without such a history [42]. Thus, how clinical outcome (i.e. development of atopy) will relate to cord blood IL-13 concentration is not entirely clear. Whether the effect of fish oil on IL-13 concentration reported here translates into a true protective effect or not can only be revealed by following these infants for an extended period of time. It is the authors stated aim to do so, and those interested in the field will await the findings with great anticipation. In the meantime, the results from this study will encourage related, well-designed, appropriately controlled and powered studies of fish oil intervention in pregnant women whose babies are at risk of developing atopic disease. In addition to studying the development of disease outcomes in the infants and children, these studies should seek to establish more precisely the cellular mechanisms by which n-3 fatty acids exert their protective effects.

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