

Does fish oil supplementation in pregnancy reduce the risk of allergic disease in infants?

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Purpose of review

Parallel increases in many inflammatory diseases over the last 40 years suggest that common environmental changes are promoting inflammatory immune responses and/or inhibiting the processes that normally keep these in check. One key change during this period has been declining intakes of anti-inflammatory dietary factors, including omega-3 polyunsaturated fatty acids (n-3 PUFA). As allergic diseases often first manifest in early infancy, prevention strategies need to be targeted early, even *in utero*. This review will examine recent evidence for the use of fish oil during this early period as a primary prevention strategy for allergic disease.

Recent findings

N-3 PUFA have well documented anti-inflammatory effects *in vitro* and have also been demonstrated to have health benefits in a range of chronic inflammatory diseases, including cardiovascular disease, rheumatoid arthritis and diabetes, supporting their role in modulating inflammation *in vivo*. Although the effects of fish oil supplementation in established allergic disease are less convincing, there is accumulating evidence that dietary n-3 PUFA may have greater effects before allergic responses are established.

Summary

Supplementation of the maternal diet in pregnancy with n-3 PUFA may provide a non-invasive intervention with significant potential to prevent the development of allergic and possibly other immune-mediated diseases.

Keywords

allergy, asthma, atopy, cord blood, cytokines, fish oil, neonate, pregnancy, primary prevention, PUFA

Introduction

Allergic diseases have become one of the major health issues in modern civilization. Up to 40–50% of the general population are now affected in many affluent countries. The increasing prevalence and the need to reverse this has led to an urgent search for the environmental changes which could be responsible. Changing dietary exposures is one major candidate factor, as both epidemiological and experimental evidence suggest a link between the declining consumption of anti-inflammatory n-3 polyunsaturated fatty acids (n-3 PUFA) with the rise in allergic diseases. Interest in a potential role of these nutrients in disease prevention has therefore been growing. Because allergic diseases often first manifest in early infancy, it is necessary to examine preventive strategies in very early life, even *in utero*. Supplementation of maternal diet in pregnancy with n-3 PUFA provides a non-invasive intervention that has significant potential to prevent the development of allergic (and, possibly, other immune-mediated diseases). This review will examine the current evidence for the role of supplementation with fish oil (rich in n-3 PUFA) in pregnancy in the prevention of allergic disease in infants.

Allergy and immune dysregulation: current perspectives

Allergic diseases are generally associated with inappropriate IgE-mediated immune responses to otherwise harmless environmental allergens. While conclusive evidence that this is due to underlying T-helper type 2 (Th2) cellular immune responses exists, the processes driving these inappropriate responses are still uncertain. It was originally proposed that allergic Th2 responses occurred as a result of 'skewing' of responses away from T-helper type 1 (Th1) responses along a Th1/Th2 continuum [1]. Therefore, great interest in cells with the capacity to influence the pattern and magnitude of T-helper cell responses, namely antigen presenting cells (APC), which provide key signals during initial antigen encounter developed. Although no conclusive evidence of primary APC defects in allergic individuals exists, environmental exposures (particularly microbial) are known to alter APC function and could potentially underlie the increasing propensity for Th2 (IL-4, IL-5, IL-9 and IL-13) responses at the population level. More recent observations challenge the Th1/Th2 paradigm, which does not explain parallel rises in both Th1 (autoimmune disease) as well as Th2-mediated diseases in the last half-century [2].

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Abbreviations

APC antigen presenting cells
PUFA polyunsaturated fatty acids
Th1 T-helper type 1
Th2 T-helper type 2

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It is also in conflict with observations that many allergic individuals also have stronger Th1 responses to allergens [3–5], and that Th1 cytokines (IL-2, IFN γ) also play a key role in mediating allergic inflammation in both atopic dermatitis [6] and asthma [7]. A new model which incorporates these observations proposes that modern environmental changes may be affecting common underlying regulatory pathways such that they are less effective in inhibiting inappropriate Th1 and Th2 responses [8]. Resulting impaired immune regulation leads to an increased risk of developing both allergic Th2 diseases and autoimmune Th1 diseases (rheumatoid arthritis, type 1 diabetes and Crohn's disease), depending on individual genetic predisposition. Consequently, intense interest in cells with regulatory properties (including APC, T-regulatory cells, epithelial cells and others) and environmental factors which may influence their function now exists. Dietary n-3 PUFA are among the main environmental changes with known immunomodulatory properties, and are therefore of central interest, defining causal pathways behind the rise in immune diseases. In addition to their therapeutic effects in many of these inflammatory diseases, a role for these fatty acids is also now being explored in prevention of disease in early life when immune responses are first initiated.

The importance of early life events and maternal exposures in infant development and disease predisposition

Early events are critical in determining subsequent development of all systems and future disease susceptibility. This is also true of the immune system. Variations in immune function in early life have been associated with increased susceptibility to allergic disease [9–13] and airways inflammation [14]. Environmental factors with the potential to influence immune development during this period are of prime interest in both understanding the epidemic rise in disease, and also as possible avenues of reversing this for disease prevention.

In addition to the well described role of n-3 PUFA in fetal neurodevelopment, these essential fatty acids are also likely to play a key role in the development of other systems including the immune system. During pregnancy, immune tolerance between the mother and the fetus is mediated by a number of complex processes at the materno–fetal interface. With well documented immunomodulatory properties, n-3 PUFA are integral in a number of these pathways. These fatty acids [particularly docosahexaenoic acid (DHA; 22:6n-3)] inhibit cell-mediated immune responses by down-regulating MHC Class II expression at the materno–fetal interface [15]. Depletion of these fatty acids, which are normally concentrated in the fetal circulation (at 10–15 times maternal levels), has been associated with pre-term birth

[16]. Another important tolerance mechanism involves a relative dominance of 'Th2' responses, which also help to prevent maternal Th1-mediated rejection of the fetal allograft [17]. This is also associated with changes in fatty acid and prostaglandin metabolism [18]. However, although n-3 PUFA are known to influence cytokine responses, their precise specific role in early immune programming is still under investigation.

These important mechanisms for modulating T-cell activity during early development emphasize the potential effect of maternal dietary fatty acids on developing immune responses to many proteins, including allergens, during this early period. The first allergen-specific responses are detectable as early as 22 weeks' gestation [19], but these do not appear to be typical 'memory' responses [20,21] and the potential mechanisms of 'priming' remain debatable [11]. Although allergens (and other antigens) induce MHC class II-dependent cord blood lymphoproliferative responses, this is associated with unusually high levels of apoptosis [22]. Furthermore, the surviving cell populations appear to have 'suppressive' or 'regulatory' properties in culture. These cells also express markers including CD4, CD25, and CTLA4 (intracellular), which are common (but not exclusive) to T-regulatory cells [22]. The significance of this is still unclear and further studies are needed. Obvious interest in the role of these cells in early regulation of immune development exists. The key issues are now to determine (1) whether there are pre-symptomatic differences in future allergic individuals, and (2) whether this is influenced by early exposures.

As this is still a very new area of research, there is no information about specific effects of n-3 PUFA on early T-regulatory pathways, but there are other associations with other aspects of immune function (as discussed further below). Furthermore, variations in neonatal n-3 PUFA status have been associated with allergic disease (in the family or in the child subsequently) [23,24]. Although this contributes to speculation that these fatty acids could have a defining role in disease pathogenesis, the evidence is still very inconclusive. Relationships between cord blood n-3/n-6 PUFA profiles and later allergy (or a family history of allergy) are variable and not predictive [25,26^{*}]. Associations between maternal breast milk n-3 PUFA levels and infant atopy are also inconclusive. Earlier studies observed that higher breast milk n-3 PUFA levels protected from infant atopy [27], whereas more recent studies [28^{*}] found the opposite (as discussed further below). Though conflicting, these and other findings do suggest an association between maternal PUFA status and allergy predisposition, and further research is clearly required to delineate this further.

Early immune development: current concepts

At birth, significantly impaired Th1 function appears due to the Th2 milieu of pregnancy and partly the result of intrinsic immaturity. In the postnatal period, this predisposition for Th2 responses persists for the first 18 months of life as Th1 responses mature gradually [10]. A number of early immunologic differences have been reported in children who later develop allergic disease [9–13,29–32]. One of the most consistent observations has been an association between relatively weaker Th1 responses in the perinatal period compared with children who do not develop allergic disease or who do not have family history of disease [30,33]. This has led to speculation that allergic disease may be the result of less efficient postnatal Th1 maturation, and a persistence of Th2 patterns of responsiveness. As yet, there is no definitive proof of this and more recent, larger longitudinal studies have observed that children with asthma, wheeze and atopy at 6 years of age also had lower levels of other cytokines (including Th2 IL-4), as well as Th1 IFN γ in cord blood [14]. It has not been clear whether these perinatal differences are merely a detectable measure of increased genetic predisposition, or whether they are indicative of early influences *in utero* that are already promoting the development of an allergic phenotype. It seems increasingly likely that both are true, particularly as some antenatal exposures (such as infection [34], maternal diet [35] and smoking [36]) have been demonstrated to modify neonatal immune function. The development of T-regulatory pathways is still being documented in this early period, but there is already intense interest in factors which could influence these pathways. Second to microbial exposures, dietary factors (including n-3 PUFA) are now being more closely investigated in this context.

In addition to their role in promoting materno–fetal tolerance in the antenatal period, n-3 PUFA status in this period may also influence the subsequent development of oral tolerance. Rodents fed a n-3 PUFA-rich diet [α -linolenic acid (ALA; 18:3n-3)] during late gestation and lactation are more likely to develop tolerance to ovalbumin (reduced delayed-type hypersensitivity and antibody responses) compared with those on a rich n-6 PUFA soybean diet [37,38[•]]. The role of n-3 PUFA in children with food allergy has not yet been determined, but remains of interest because many of these children go on to develop persistent aeroallergens sensitization and associated respiratory disease.

The effects of n-3 PUFA on immune function: recent evidence

The antiinflammatory properties of n-3 PUFA have been recently reviewed [39,40,41^{••},42^{••}]. As a vital structural component of the cell membrane, n-3 PUFA are able to modulate cell membrane fluidity and affect cell

signaling. These fatty acids are also the precursors for the synthesis of eicosanoids which have potent effects on immune responses. Eicosanoids, derived from the oxidative metabolism of arachidonic acid (AA; 20:4n-6), are important mediators in the development of airways inflammation in asthma (reviewed [43]). Metabolism of AA via the cyclooxygenase pathway results in the production of prostaglandins (PG)E₂ and D₂ and thromboxanes, whereas metabolism via the 5-lipoxygenase pathway produces leukotriene (LT) B₄ and the cysteinyl leukotrienes C₄, D₄, and E₄. These are potent mediators of bronchoconstriction, vascular permeability and mucus secretion in specific organs. Supplementation with fish oil [rich in eicosapentaenoic acid (EPA; 20:5n-3) and DHA] reduces the amount of AA available for biosynthesis of LTB₄ [44] and other pro-inflammatory mediators, as n-3 and n-6 PUFA compete for common desaturation enzymes. It has also recently been demonstrated that DHA and EPA also produce novel anti-inflammatory lipid mediators, resolvins and docosatrienes which are released from neutrophils [45[•]].

A plethora of recent papers reporting health effects of n-3 PUFA on a range of chronic inflammatory disorders has stimulated much interest in cell signaling as a common mechanism for these effects. New data suggest that n-3 PUFA membrane composition alters the functionality of lipid microdomains (lipid rafts), which mediate cellular responses in a variety of tissues [46[•]]. Dietary DHA has also been associated with suppression of protein kinase C and IL-2 production in a mouse model [47], resulting in reduced lymphoproliferation. Similarly, T-cell apoptosis, mediated by raft structure, is also influenced by n-3 PUFA [48].

In another study, human subjects consuming fish oil show significant suppression of cyclooxygenase-2 expression in blood monocytes when stimulated *in vitro* with lipopolysaccharide, an agonist for Toll-like receptor 4. This shows a novel link between fatty acid and microbial signaling pathways [49]. The role of n-3 PUFA in mediating these effects has recently focused on effects on gene expression using micro-array analysis [50^{••}]. In particular, effects of n-3 PUFA on transcription factors such as SREBP1c and nuclear receptors such as PPAR- α , HNF-4 α and LXRA α are being addressed [51^{••}].

Links between n-3 long-chain PUFA and established allergic disease

Some disparity between data from cross-sectional and intervention studies which have examined links between n-3 PUFA and allergic disease exists. Meta-analyses of randomized controlled trials have generally shown limited beneficial effect of fish oil supplementation in adults with established asthma [52] or atopic dermatitis [53[•]]

and there is a lack of data to conclude effectiveness in young atopic children. In contrast, data from cross-sectional studies have provided stronger evidence for a link between n-3 fatty acid intake and the risk of allergic disease, particularly in children. In a recent prospective study of 335 Australian children aged 6–8 years (in which 49% had current asthma), there was a significant association between an increased ratio of n-6:n-3 fatty acid and risk of current asthma [54^{••}]. In another study, the ratio of n-6 AA:n-3 EPA at birth was positively associated with eczema at 18–30 months (adjusted OR per doubling = 1.14, 95% CI 1.00–1.31, $P = 0.044$) and the ratio of linoleic acid (LA; 18:2n-6):ALA was positively associated with later-onset wheeze at 30–42 months (OR = 1.30, 95% CI 1.04–1.61, $P = 0.019$) [26[•]]. These data provide evidence that, at least in children, a diet rich in n-3 PUFA may protect against symptoms of asthma, and has provided further support for examining the role of early supplementation for disease prevention (as discussed further below).

While the role of breastfeeding in the prevention of allergic disease is controversial, there is a substantial body of evidence suggesting that breast-feeding does afford some protection from allergy (discussed in [55]). Although it is not clear which components are responsible for the potentially protective effects, there is evidence linking breast-milk fatty acid composition and atopic outcomes [56,57]. Breast milk provides essential nutrients, including dietary fatty acids as well as many immunomodulatory factors (soluble CD14, IgA and cytokines) with the potential to influence infant immune development and oral tolerance. However, not all studies have shown beneficial effects of breast-milk n-3 PUFA composition on allergy outcome. Stoney *et al.* [28[•]] recently reported that maternal colostrum levels of docosapentaenoic acid (DPA; 22:5n-3) and DHA were significantly higher in infants sensitized to food at age 6 months ($P = 0.001$ and $P = 0.007$, respectively) than non-sensitized infants. Immune parameters were not reported in this study. In another study, atopic women had reduced TGF β 2 concentration in the breast milk compared with non-atopic mothers [58], suggesting additional potential effects of breast milk on the developing mucosal immune system. However, although it appears possible to influence the immunological properties of breast milk by dietary intervention, the clinical benefits appear small at best and the long-term effects are yet to be determined.

Intervention with n-3 PUFA in the perinatal period for the prevention of allergic disease

We and others have proposed that dietary n-3 PUFA have greater effects on immune function early in life, while responses are still developing. This may explain the more debatable effects of n-3 PUFA in adults with established

disease. To date, there have been only two studies which have used fish oil supplementation for primary prevention of allergic disease. The first of these was a randomized control trial [59], which examined the effects of 500 mg/day fish oil (versus placebo) in infants at high risk of allergic disease as soon as they were weaned (or at 6 months of age, if breast-fed). At 18 months of age, fish oil supplementation was associated with a 9.8% reduction (95% CI 1.5–18.1, $P = 0.02$) in wheeze and a 7.8% absolute reduction (95% CI 0.5–15.1, $P = 0.04$) in prevalence of wheeze for more than one week [59,60^{••}]. At age 3 years, there was a significant 10% reduction (95% CI 3.7–16.4, $P = 0.003$) of cough in atopic but not non-atopic children [61^{••}]. No difference in total IgE concentration between the two groups, however, existed, suggesting that intervention may need to occur before 6 months of age to prevent early sensitization.

In a second study, we examined the effects of a much earlier intervention (in pregnancy) on neonatal immune responses. In this double-blind, controlled trial, 98 allergic women were randomized to receive fish oil (3.7 g n-3 PUFA/day) or control (olive oil) capsules from 20 weeks' gestation until delivery [35,62,63^{••},64^{••}]. Eighty-three women and their healthy babies completed the study. The primary objectives were to determine the effect of n-3 PUFA supplementation on neonatal PUFA levels, cytokine levels and immunological response to allergens and other stimuli. Maternal fish oil supplementation was associated with a consistent trend for attenuated Th1 [IFN γ and Th2 cytokine (IL-5, IL-13 and IL-10)] response to allergens. In particular, there was a significant reduction in IL-10 production but no overall modification of the ratio of Th1:Th2 cytokine responses. Furthermore, neonates whose mothers received fish oil had significantly lower levels of circulating Th2 IL-13 in cord blood compared with the control group. A significant inverse relationship between levels of n-3 PUFA in neonatal cell membranes and plasma IL-13 also existed. Although clinical development of atopy was evaluated as a secondary outcome, at 1 year of age, infants supplemented prenatally with fish oil were three times less likely to have a positive skin-prick test to egg (OR = 0.34, 95% CI 0.11–1.02, $P = 0.05$). Infants with eczema at 1 year had significantly fewer severe symptoms (OR = 0.09, 95% CI 0.01–0.94, $P = 0.045$) if their mothers were supplemented with fish oil. We also showed that maternal fish oil supplements in pregnancy significantly altered breast-milk PUFA levels [63^{••}]. Furthermore, n-3 PUFA levels were positively associated with IgA and sCD14, as well as cytokines involved in the synthesis of IgA (IL-6 and IL-10), suggesting a relationship between fatty acid status and mucosal immune function.

Fish oil supplementation in pregnancy [65] and in the first year of life [66] also appears to reduce the risk

of other (Th1) mediated immune diseases such as childhood-onset type 1 diabetes. This apparent benefit in both Th1 and Th2-mediated diseases supports the notion that changes in immunomodulatory environmental factors such as n-3 PUFA may exert their effects through common regulatory pathways rather than through simple Th1/Th2 skewing effects. Further studies are now needed to confirm the clinical benefits of n-3 PUFA in early life and to define the underlying mechanisms of action.

Future directions

'Natural' safe strategies to prevent disease have a very high public appeal; however, more studies are clearly needed before recommendations can be made for n-3 PUFA in allergy prevention. If benefits are confirmed, the timing, duration and dosage of supplementation need to be determined. Other prevention strategies are also being investigated and it is possible that future strategies could include combinations of n-3 PUFA, antioxidants and probiotics. Ideally, dietary prevention strategies would be achievable using fresh foods; however, the rising levels of pollution in global fish stocks has become of increasing concern. Fish oils are also susceptible to ubiquitous contaminants such as lipophilic persistent organic pollutants such that levels must be carefully monitored [67]. Alternative sources of short-chain n-3 PUFA ALA are found in vegetable oils; however, the efficiency of conversion of elongation and desaturation to EPA and, in particular, DHA is reduced. Recent data indicate the potential for the use of transgenic plants and reverse engineering for the production of EPA (but not DHA, as yet) [68]. This technology could lead to the development of new anti-inflammatory agents by chemically engineering PUFA. A novel n-3 PUFA (β -oxa 21:3n-3) that has more selective immunologic effects (stronger inhibition of T-cell activity than native n-3 PUFA but without associated stimulation of the neutrophil respiratory burst) has been engineered [69]. While no current therapeutic role for this or other such compounds exists, this illustrates another potential future avenue for approaching prevention and therapy of inflammatory diseases.

Conclusion

More definitive prevention strategies depend on a better understanding of (1) how allergic immune responses develop in early life; (2) which environmental changes are responsible for the recent increase in allergic disease; (3) how these factors influence the developing immune system; and (4) how these or other factors can be modified effectively to reverse this process without adverse effects.

Dietary supplementation with n-3 PUFA in pregnancy (or early life) provides an important potential strategy to prevent immune-mediated disease early in life. The data

presented here provide strong evidence that n-3 PUFA play a key role in regulating immune responses in early life particularly in pregnancy when materno-fetal alloantigen tolerance is critical. It is logical that this immunomodulation could extend to other antigens encountered during this period (including allergens). This is supported by studies by our group that n-3 PUFA supplementation can alter neonatal immune responses to allergens. Preliminary evidence from this and other studies that higher levels of dietary n-3 PUFA (and/or lower levels of n-6 PUFA) during early critical periods of development can protect against allergic disease [35,54,59] also exists. Larger longitudinal intervention studies are now clearly needed.

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