Scientific Review: The Role of Nutrients in Immune Function of Infants and Young Children

Emerging Evidence for Long-chain Polyunsaturated Fatty Acids

Edited by

PHILIP CALDER, PhD

SUSAN PRESCOTT, MD, PhD

MICHAEL CAPLAN, MD
Preface

Mead Johnson & Company, the maker of Enfamil LIPIL® infant formulas, continues to investigate the effects of LCPUFA on health. This monograph reviews the role of nutrients in immune function of infants and young children, including a more in-depth look at the roles of LCPUFA. The intent of the monograph is to provide health care professionals a balanced overview of research on this topic. It is to be used as an educational resource only and is not intended for product support or for the development of product claims.

We are pleased that you are interested in this emerging area of nutrition and we welcome this opportunity to help you learn more about the roles of LCPUFA in nutrition.
Table of Contents

Executive Summary 1

Introduction 5

LCPUFA in Health: An Overview 6

Immunology and the Immune System: An Overview 11

Effects of LCPUFA on Immune Function 16

LCPUFA and Healthy Immune System Development: Clinical Reports in Pregnancy, Lactation, and Early Childhood 20

Overall Summary and Conclusions 27

References 29
Executive Summary

Growing evidence suggests that long-chain polyunsaturated fatty acids (LCPUFA) have critical roles in the growth and development of infants and children and may have beneficial long-term effects on health throughout life. Studies have shown that two LCPUFA in particular, docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6), have important roles in infant cognitive development, visual acuity, and growth. These two LCPUFA are naturally present in human milk and are permitted as supplemental ingredients in infant formulas available in many countries. The purpose of this monograph is to review published studies evaluating the roles of dietary LCPUFA in supporting immune system development and function.

Widespread changes in dietary fat intake have occurred over the past 150 years in most industrialized nations, marked by a decrease in consumption of marine- and plant-derived n-3 fatty acids and an increase in consumption of n-6 fatty acids. As intake of n-6 fatty acids has increased, the ratio of dietary n-6:n-3 fatty acids has shifted substantially. Concomitant with changes in dietary LCPUFA intake have been significant increases in the prevalence of atopic disease in a number of populations. Some researchers have focused on dietary fatty acid intake as a potential factor in the increased prevalence of atopy.

Because atopic sensitization tends to occur in early life, clinical studies evaluating the relationship between LCPUFA intake and incidence of atopic disease in infants and children have focused in two areas:

- The effects of maternal supplementation during pregnancy and lactation on breast milk LCPUFA content and neonatal blood LCPUFA levels, and correlations with atopic disease in infants, and
- The relationship between LCPUFA intake during infancy and childhood and subsequent development of atopy.

Some epidemiological studies and preliminary clinical studies have suggested that consumption of n-3 LCPUFA in pregnancy, lactation, and early childhood may have implications for immune development and the subsequent risk of asthma and atopic disease.
LCPUFA in Health: An Overview

There is evidence from clinical studies that LCPUFA have key functions in maintaining health through their effects on the immune system and, in particular, through their modulation of the inflammatory response, which appear to be mediated through effects as precursors of inflammatory mediators, as well as through other complex cellular mechanisms. These immunomodulatory effects have generated obvious interest in the potential role of LCPUFA in common inflammatory conditions in childhood, such as asthma, eczema, atopic dermatitis, and food hypersensitivities. Epidemiological surveys support a protective role of oily fish, known to be naturally rich in n-3 LCPUFA, against atopic disease and reactive airways. A number of intervention studies using fish oil supplementation in early life are currently underway, as discussed later.

There is also accumulating evidence that supplementation of infant formula with preformed LCPUFA, particularly DHA, has positive effects on visual and mental development in preterm infants and term infants.

Sources of LCPUFA

LCPUFA can be supplied in the diet, from tissue stores, or by de novo synthesis from precursor fatty acids. Infants who are breastfed obtain LCPUFA postnatally from human milk. LCPUFA concentrations in human milk vary widely. Pregnant women who consume diets containing little fish or meats may have inadequate LCPUFA intake to meet demands for infant LCPUFA accretion and replenishment of their own LCPUFA stores. Formula-fed infants who receive formula without preformed LCPUFA must rely on endogenous synthesis from essential fatty acid precursors to meet their LCPUFA needs. Many experts now agree that rates of DHA synthesis from alpha-linolenic acid typically provided in neonatal formula are variable from infant to infant and may not provide adequate tissue DHA availability required for optimal neural development. These unpredictable systemic DHA levels may further contribute to altered immune development at a critical timepoint during infancy and early childhood.

Immunology and the Immune System Overview

Breast milk intake is a key factor in the immune system development of newborns and infants, and breast milk contains numerous components that are thought to modulate immunological responses, such as cytokines, growth factors, lactoferrin, oligosaccharides, leukocytes, IgA, and polyunsaturated fatty acids. Clinical studies suggest that increased n-3 LCPUFA concentrations in breast milk are associated with increased breast milk levels of IgA and some cytokines. The effects of such alterations in breast milk content on immune development have not yet been determined. However, it has been speculated that maternally derived immune factors could have direct immunomodulatory effects and/or promote infant IgA production.
Effects of nutrition on immune function

Appropriate functioning of the immune system is dependent on nutritional status. Malnutrition, including protein-energy malnutrition and micronutrient deficiencies, is an important risk factor for illness and death, particularly among pregnant women, infants, and young children. Poor protein intake is associated with significantly impaired immunity. Inadequate levels of vitamins and minerals in the diet can have significant negative consequences for immune function. Key vitamins evaluated for their roles in immunity include the fat-soluble vitamins A and E and the water-soluble vitamins C, B6, B12, and folate. Of the many minerals with functions in immune cell response, iron, zinc, and selenium have been the primary focus of population-based supplementation studies.

Effects of LCPUFA on Immune Function

Long-chain fatty acids, particularly DHA and ARA, are preferentially incorporated into cell membrane phospholipids. In cell membranes, LCPUFA contribute to membrane fluidity, have roles in signal transduction and gene expression, and provide substrate for production of chemical mediators. All three functions contribute to immune cell responses.

Precursors of eicosanoids and docosanoids

Chemical mediators derived from LCPUFA include eicosanoids, a family of mediators which includes prostaglandins, leukotrienes, and thromboxanes, as well as resolvins, docosatrienes, and neuroprotectins. ARA is a precursor of the 2-series prostaglandins (eg, PGE,) and thromboxanes and 4-series leukotrienes, all of which are predominantly pro-inflammatory eicosanoids. DHA can be retroconverted to eicosapentaenoic acid (EPA; 20:5n-3), which is metabolized to form the 3-series prostaglandins (eg, PGE,) and thromboxanes, as well as the 5-series leukotrienes. These EPA-derived chemical mediators tend to be less inflammatory than those produced from ARA. Both EPA and DHA give rise to resolvins and related mediators that have potent anti-inflammatory activity.

Early studies of LCPUFA effect on immune function

Numerous studies in adults and children suggest relationships between intake of specific LCPUFA and alterations in markers of immune function. Results have shown that:

- Persons with allergy may have altered levels and ratios of n-3 and n-6 fatty acids, including DHA and ARA, compared with non-allergic persons.
• Supplementing the diet with preformed LCPUFA alters the levels of those LCPUFA in plasma phospholipids and immune cells.4,24,28,29

• Supplementing the diet with preformed LCPUFA results in discrete changes in immune cell and cytokine production; these changes differ with type of LCPUFA supplementation and are influenced by the ratio of n-6 to n-3 LCPUFA in the diet.30,31

LCPUFA and Healthy Immune System Development: Clinical Reports in Pregnancy, Lactation, and Early Childhood

There is a growing body of evidence suggesting that dietary intake of LCPUFA early in life could influence immune development and other health outcomes, including respiratory health. Because maternal diet is the most important determinant of LCPUFA accretion for the woman and her infant, dietary investigations have focused on the relationship between LCPUFA intake and status in pregnant and breastfeeding women and the incidence of atopic diseases in their children. Numerous studies have demonstrated relationships between breast milk LCPUFA composition and incidence or markers of atopic disease in infants and children. Several studies have shown relationships between maternal LCPUFA intake and development of atopic dermatitis in breastfed infants, although these relationships are not consistent.

Emerging evidence in both animal and human studies suggests that n-6 LCPUFA, including ARA, have important roles in immune system development and regulation.29,32,33 Data from animal studies have demonstrated a substantial accretion of ARA in the thymus during early growth and development,32 and ARA is consistently present in breast milk.

There are now a number of studies investigating the role of n-3 LCPUFA supplementation in early life in the development and progression of atopy and childhood asthma. For example, studies have examined the relationships between maternal and infant intake of n-3 LCPUFA, immune development, and subsequent development of atopy. At this stage there is only preliminary evidence that exposure to adequate n-3 LCPUFA in utero and via breast milk is associated with reduced development of atopic disease in infants and children.34 Further studies are underway to confirm these associations. Several studies are also underway to evaluate the effects of early postnatal fish oil supplementation on asthma and allergic outcomes. The only findings published so far indicate no clear benefits on these outcomes at 5 years of age,10 although there were benefits at 184 and 367 months of age. A number of other studies are on-going which will assess the effects of supplementation with higher doses of n-3 PUFA from birth.
Growing evidence in animal and human studies suggests that long-chain polyunsaturated fatty acids (LCPUFA) have critical roles in the growth and development of infants and children and may have beneficial long-term effects on health throughout life. Studies have shown that two LCPUFA in particular, docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6), have important roles in infant cognitive development, visual acuity, and growth. These two LCPUFA are naturally present in human milk and are provided as supplemental ingredients in many infant formulas available around the world. There is clinical evidence that infants may benefit from preformed dietary DHA and ARA either in breast milk or in supplemented infant formulas. Furthermore, certain LCPUFA function as precursors of compounds involved in modulation of the immune response. The purpose of this monograph is to review published studies evaluating the roles of dietary LCPUFA in supporting immune system development and function.

Widespread changes in dietary fat intake have occurred over the past 150 years in most industrialized nations, marked by a decrease in marine- and plant-derived n-3 fatty acids and an increase in consumption of n-6 fatty acids.\(^1\),\(^2\) As intake of n-6 fatty acids has increased, the ratio of dietary n-6:n-3 fatty acids has shifted substantially. Whereas “traditional” diets of early humans provided a ratio of 1-2:1, contemporary diets provide a ratio up to 30:1.\(^2\) Population-based intake data used to establish Dietary Reference Intake (DRI) levels of n-6 and n-3 fatty acids suggest that this ratio is approximately 10:1 in US diets.\(^3\)\(^8\)

Concomitant with changes in dietary LCPUFA intake have been significant increases in the prevalence of atopic disease in a number of populations.\(^3\) The rapidity of this rise suggests that environmental factors, including alterations in dietary fat intake, may be responsible.\(^3\)\(^9\),\(^4\)\(^0\) Because atopic sensitization tends to begin in early life,\(^4\)\(^1\),\(^4\)\(^2\) clinical studies evaluating the relationship between LCPUFA intake and incidence of atopic disease in infants and children have focused in two areas:

- The effects of maternal supplementation during pregnancy and lactation on breast milk LCPUFA content and neonatal LCPUFA levels, and correlations with atopic disease in infants, and
- The relationship between LCPUFA intake during infancy and childhood and subsequent development of atopy.

Current understanding of the effects of maternal LCPUFA supplementation in pregnancy is only preliminary, but suggests early immune effects and reduced allergic outcomes.\(^2\)\(^8\),\(^4\)\(^3\) Further studies are underway. The reported effects of postnatal supplementation have been disappointing to date, with no long-term benefits of fish oil supplementation in early infancy on asthma or allergy outcomes at 5 years of age.\(^3\)\(^5\) The results of other postnatal intervention studies currently underway are awaited with interest.
There is evidence that LCPUFA have key functions in maintaining health. Clinical studies have shown that dietary LCPUFA may have positive effects on visual and mental development, atopic disease, and intestinal health, and may influence cardiovascular disease. LCPUFA are structural components of cell membranes and are precursors of other compounds which influence the inflammatory response. Inflammation is a key component of the normal immune response, yet inappropriate or uncontrolled responses can result in disease. LCPUFA and metabolites of LCPUFA influence systemic immune responses to surgery, injury, and infection, as well as chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

Positive influence on visual and mental development

It has been well-established that supplementation of infant formula with preformed LCPUFA, particularly DHA, has positive effects on visual development in preterm infants and term infants. DHA supplementation of infant formula has also been associated with improved measures of mental development in preterm infants and term infants. Among breastfed infants, increased blood DHA levels have been associated with improvements in neural and visual development.

Possible immune effects

Conditions commonly associated with atopy, such as asthma, wheezing, eczema, and food hypersensitivities, are common in children. These conditions have a strong inflammatory component and could therefore be influenced by dietary LCPUFA. Epidemiological surveys support a protective role of oily fish, known to be naturally rich in long-chain n-3 polyunsaturated fatty acids, against asthma. Data from the first US National Health and Nutrition Survey (NHANES I) found that dietary fish intake was positively associated with lung function in adults. An Australian study noted that children who regularly consumed oily fish had a significantly reduced risk of current asthma. Some recent prospective intervention and observational studies have provided support for a role of LCPUFA in the development of immune function and atopic disease in young children, whereas others have not.

LCPUFA appear to modulate immune effects through a number of complex pathways, most notably as precursors of eicosanoids and through effects on intracellular signaling, which affects many aspects of cell function (including cytokine production and cell activation molecules). LCPUFA may also have secondary effects by modifying oxidative stress. Dietary LCPUFA are preferentially incorporated into cellular membranes, where their highly unsaturated chemical structure lends fluidity to the membranes, and where they provide a reservoir for eicosanoid production. Thus, LCPUFA can affect broad-ranging aspects of immune cell function. Further studies are needed to determine the relevance of these effects during pregnancy and early childhood.
Positive influence on intestinal health

In vitro, animal, and preliminary clinical studies suggest that LCPUFA may have a role in promoting intestinal health. LCPUFA have been shown to influence gut barrier function by affecting intestinal secretions, mucus secretions, and density of surfactants in the mucus layer. In vitro studies have shown that specific fatty acids, including LCPUFA, may influence gut microflora by inhibiting growth of pathogenic bacteria (anaerobes). It is well-established that the immature infant gut is susceptible to antigen sensitization, and that early feeding experiences can influence development of food allergies. Gut-associated lymphoid tissue and numerous immune cells in the intestine provide a primary defense against food-borne antigens and pathogens. Interventions which increase the strength of this defense may reduce infants’ risk of atopic sensitization.

Infants born prematurely are particularly at risk for necrotizing enterocolitis (NEC), an inflammatory disorder of the intestine which is associated with significant morbidity and mortality. In a double-blind, randomized, controlled study of LCPUFA supplementation in preterm infants, it was found that infants fed preterm formula providing higher levels of ARA, DHA, and esterified choline developed significantly less NEC compared with infants fed a control formula (2.9% vs 17.6%). A neonatal rat model also showed that feeding LCPUFA reduced the incidence of necrotizing enterocolitis (NEC) and intestinal inflammation. Additional clinical studies, however, showed no significant benefit of LCPUFA on the incidence of NEC in preterm infants, but differences in study design, dosing, and appropriate numbers of enrolled infants preclude a clear conclusion on this potentially interesting relationship and further experimental investigation is warranted.

Potential influence on cardiovascular health

LCPUFA also are being investigated for their role in the prevention of endothelial activation, with implications for the initiation, progression, and clinical course of atherosclerosis. Preliminary clinical studies suggest that LCPUFA consumption early in life may have lasting effects on cardiovascular risk; infants who received formula with added DHA and ARA for the first 4 months of life had lower blood pressure at 6 years of age compared with infants who received unsupplemented formula although both groups were within the normal range.

Sources of LCPUFA

LCPUFA can be supplied as a component of the diet, from tissue stores, or by de novo synthesis from precursor fatty acids. LCPUFA are naturally present in most animal tissues. n-3 LCPUFA, including DHA and eicosapentaenoic acid (EPA; 20:5n-3), tend to be higher in
fish and shellfish than in other animal products, and are particularly high in fatty fish (Table 1). Although these foods are not recommended for young infants, they do provide dietary sources of LCPUFA for pregnant and lactating women. Human milk contains preformed LCPUFA in varying amounts, which can be affected by the fat composition of the maternal diet. In a study of pregnant women in Canada, fish and shellfish contributed approximately 80% of dietary DHA while meat and poultry contributed approximately 80% of dietary ARA. Eggs contributed smaller amounts of both DHA and ARA to subjects’ diets.

Table 1. Selected Food Sources of LCPUFA

<table>
<thead>
<tr>
<th>Food Source</th>
<th>DHA (mg)</th>
<th>EPA (mg)</th>
<th>ARA (mg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon filet, baked/broiled (3 oz)</td>
<td>638</td>
<td>456</td>
<td>85</td>
</tr>
<tr>
<td>Mackerel, baked/broiled (3 oz)</td>
<td>594</td>
<td>428</td>
<td>43</td>
</tr>
<tr>
<td>White tuna, canned in water (3 oz)</td>
<td>535</td>
<td>198</td>
<td>43</td>
</tr>
<tr>
<td>Crab, steamed (3 oz)</td>
<td>196</td>
<td>207</td>
<td>71</td>
</tr>
<tr>
<td>Chicken, roasted, dark meat (1 cup)</td>
<td>70</td>
<td>14</td>
<td>196</td>
</tr>
<tr>
<td>Chicken, roasted, light meat (1 cup)</td>
<td>42</td>
<td>14</td>
<td>98</td>
</tr>
<tr>
<td>Turkey, roasted, dark meat (1 cup)</td>
<td>84</td>
<td>0</td>
<td>364</td>
</tr>
<tr>
<td>Turkey, roasted, light meat (1 cup)</td>
<td>42</td>
<td>0</td>
<td>210</td>
</tr>
<tr>
<td>Egg, hard-boiled (1 large)</td>
<td>19</td>
<td>3</td>
<td>74</td>
</tr>
</tbody>
</table>

*Data for ARA are reported as “20:4 undifferentiated” by the USDA.

Prior to birth, infants can obtain LCPUFA directly from their mothers via placental transfer. LCPUFA, and DHA in particular, accrue in fetal adipose tissue with advancing gestational age and may serve as a reservoir for LCPUFA after birth. Studies have consistently shown a significant association between maternal and newborn infant DHA and ARA concentrations. Because much of the infant’s tissue LCPUFA accumulation occurs in the third trimester of pregnancy, preterm infants are born with lower total LCPUFA reserves compared with term infants.

**LCPUFA sources: breastfed infants**

Postnatally, infants who are breastfed obtain LCPUFA from human milk. LCPUFA concentrations in human milk vary widely. Numerous studies conducted among women in different
geographic regions have evaluated and quantified human milk levels of LCPUFA. A review of studies from European and African countries identified median human milk DHA levels of 0.3% of fatty acids and median ARA levels of 0.5% to 0.6% of fatty acids.62 Another review of worldwide human milk LCPUFA levels reported mean DHA content of 0.45% to 0.88% of fatty acids and ARA content of 0.42% to 0.92% of fatty acids.61 Worldwide average concentrations of DHA and ARA in human milk can be estimated at 17 mg DHA/100 Cal and 34 mg ARA/100 Cal (based on 0.3% of fatty acids as DHA and 0.6% as ARA, and 50% of calories from fat).60-62

Pregnant and lactating women who consume diets containing little fish or meat may have inadequate LCPUFA intake to meet demands for infant LCPUFA accretion and for replenishment of their own LCPUFA stores.17 Dietary supplements are available containing n-3 LCPUFA derived from single-cell organisms or from fish oil which has been processed to remove most or all potential contaminants.

**LCPUFA sources: formula-fed infants**

Formula-fed infants who receive formula without preformed LCPUFA must rely on tissue stores and endogenous synthesis from essential fatty acid precursors to meet their LCPUFA needs (Figure 1).18 Through a series of desaturation and elongation reactions, enzymes present in the liver and other tissues can convert linoleic acid and alpha-linolenic acid into long-chain derivatives. Linoleic acid is converted to ARA, which may be incorporated into membrane structures or metabolized further into specific eicosanoids. Alpha-linolenic acid is converted to EPA and to DHA; EPA is used to produce a different series of eicosanoids. DHA and EPA are also precursors of chemical mediators called resolvins, docosatrienes, and neuroprotectins.23,68 Because n-3 and n-6 fatty acids compete for enzymes involved in LCPUFA metabolism and conversion to other compounds, an appropriate dietary balance of n-3 vs n-6 fatty acids is important. Animal studies have shown that the n-3 fatty acid alpha-linolenic acid is a strong suppressor of n-6 fatty acid conversion to LCPUFA; the n-6 fatty acid linoleic acid is less able to suppress n-3 fatty acid conversion to LCPUFA.47

Many experts now agree that rates of DHA synthesis from alpha-linolenic acid at levels typically provided in neonatal formula are variable from infant to infant19 and may not provide adequate tissue DHA availability required for neural development.20 These unpredictable systemic DHA levels may further contribute to altered immune development at a critical timepoint during infancy and early childhood. Infants appear to differ in the extent to which they can convert alpha-linolenic acid into n-3 LCPUFA69 and in the rates at which newly formed n-3 LCPUFA are incorporated into plasma phospholipid.70 Further, because alpha-linolenic acid may be preferentially oxidized for energy,71,72 its availability for conversion to n-3 LCPUFA may be limited in some infants despite apparently adequate intakes.69 Some expert organizations have called for the inclusion of preformed DHA and ARA in term and preterm infant formulas.73-77 Commercial infant formulas containing DHA and ARA are available in many markets.
Figure 1. Conversion of Essential Fatty Acids to LCPUFA

Adapted from Innis\(^\text{18}\) and Carlson.\(^\text{66}\)
The immune system must achieve a fine balance between providing “protective surveillance” against threats, including infectious agents and malignancy, while at the same time maintaining “tolerance” to harmless things, such as “self” and other commonly encountered harmless environmental factors.

Allergy results when there is a breakdown in normal “tolerance” mechanisms, which leads to inappropriate and detrimental immune responses to normally harmless substances, including food allergens such as cow’s milk protein, eggs, nuts, or shellfish, and environmental allergens such as mold, dust mites, or pollen. Allergic reactions typically result in one or more of a group of symptoms usually involving mucosal or cutaneous surfaces where environmental factors are first encountered, including atopic dermatitis (eczema), asthma, wheezing, coughing, rhinitis, sneezing, nasal congestion, vomiting, and diarrhea.

The immune system is made up of an integrated network of organs, tissues, cells, and molecules which work together to resist infection while maintaining tolerance to harmless factors such as “self” antigens and allergens. When a challenge is detected (ie, an allergen or pathogen), cell signaling within and between immune cell types allows a coordinated immune response. At the cellular level, this involves the secretion of multiple cytokines and eicosanoids, which in normal situations enable cells to communicate with each other and to bind, neutralize, and eliminate the challenge.

At birth, the immune system is immature, but it develops with age, antigen stimulation, and appropriate nutrition. In addition, bacterial colonization occurs during the first weeks of life, and interactions between intestinal flora and the developing mucosa result in further development of immune responses and oral tolerance. Nutrition plays a key role in the development, maintenance, and optimal functioning of immune cells. Dietary nutrients such as DHA, EPA, and ARA influence the nature of an immune response by modulating the types and amounts of cellular messengers secreted and by reducing oxidative stress.

Breast milk intake appears to be a key factor in immune system development among neonates and infants. Clinical studies suggest that increased n-3 LCPUFA concentrations in breast milk are associated with increased breast milk levels of IgA and some cytokines. The effects of these alterations in milk content on immune development have not yet been determined. However, it has been speculated that maternally derived immune factors could have direct immunomodulatory effects and/or promote infant IgA production. Immune factors provided to the infant in breast milk, including antigen-specific IgA, cytokines, and other immune cells, may contribute to the preventive effects of breastfeeding on allergy, although this has not been confirmed.
Effects of nutrition on immune function

Overall nutritional status is closely linked to appropriate functioning of the immune system. In general, deficiency of several nutrients will lead to impaired immune responses, and replenishment of those specific components will typically restore the affected responses. Consuming excessive amounts of some nutrients can also be detrimental to immune responses. Worldwide, malnutrition is closely linked to infectious diseases that are responsible for substantial morbidity and mortality among infants and children. Studies in both developing and developed countries have focused on the effects of dietary protein, dietary fat (including n-3 fatty acids), the fat-soluble vitamins A, D, and E, and minerals such as iron, selenium, and zinc. No single biomarker is available that accurately reflects the effect of nutritional interventions on the immune response; as a result, clinical outcomes are most important in determining nutrient effect.

Malnutrition

Scientists have observed the close relationship between infectious disease and malnutrition for more than 50 years. Malnutrition is an important risk factor for illness and death, particularly among pregnant women, infants, and young children. Being malnourished increases susceptibility to infection and severity of infection through multiple interacting pathways, which can lead to further deterioration of an individual's nutritional status. Protein-energy malnutrition, including marasmus (starvation) and kwashiorkor (protein deficiency), is a major cause of immunodeficiency and is associated with significant morbidity and mortality from infectious diseases. A 2005 review of literature estimated that malnutrition is indirectly responsible for about half of all deaths in young children worldwide and that the risk of death is directly correlated with the degree of malnutrition. Deficiencies in iron, zinc, vitamin A, and iodine also impact immune function and susceptibility to infection among women and children in developing countries.

Protein intake

Inadequate protein intake leads to suboptimal tissue repair and reduced resistance to infection. Poor protein intake is associated with significantly impaired immunity, as

---

Definitions

**Cytokines:** protein molecules that enable immune cells to communicate with each other in generating an immune response.

**Eicosanoids:** biologically active substances derived from precursor LCPUFA with 20-carbon chain lengths (ie, ARA [an n-6 LCPUFA] and EPA [an n-3 LCPUFA]). Examples include prostaglandins, leukotrienes, and thromboxanes.
evidenced by altered immune responses and deficits in phagocyte function, complement cascade, antibody concentrations, and cytokine production. The amino acids arginine and glutamine have been studied extensively for their roles in promoting immune response following surgery, trauma, and sepsis; studies suggest that in certain populations these amino acids may enhance wound healing, increase resistance to infection and tumorigenesis, and improve immune function.

**Nucleotides**

The effect of preformed nucleotides on immune function has been evaluated in a limited number of studies, but clinical data are inconclusive. Infants fed a nucleotide-supplemented formula had higher serum concentrations of IgA throughout a 48-week study compared to infants fed a control formula. The supplemented infants had a lower risk of diarrhea between 8 and 28 weeks, but this difference was not statistically significant over the period of 48 weeks. Supplemented infants had an increased risk of upper respiratory infections, similar incidence of lower respiratory infections, and similar antibody response to vaccination compared to unsupplemented infants. Another study suggested that nucleotide supplementation improved HiB and diphtheria antibody responses following immunization. Nucleotides may become conditionally essential during periods of growth or immunological challenge, and there has been a call for further research to define the importance of these nutrients, particularly in feeding situations involving single sources of nutrition.

**Vitamins and minerals**

Inadequate levels of certain vitamins and minerals in the diet can have significant negative consequences for immune function. Much of what is known about the roles of vitamins and minerals in immune response has come from animal studies, where control of individual dietary components is easily accomplished. Population-based studies in countries where nutrient deficiencies are endemic have substantially increased our understanding of the effects of vitamins and minerals on the human immune response. Such studies have evaluated the impact of large-scale supplementation programs on morbidity and mortality in vulnerable populations—frequently infants and young children. While such studies do not directly show causation, they provide compelling evidence of nutrient effects on immune function.

Key vitamins evaluated for their roles in immunity include the fat-soluble vitamins A and E and the water-soluble vitamins C, B6, B12, and folate. Effects of these micronutrients on immune function are summarized briefly in Table 2. Of the many minerals with functions in immune cell response, population-based studies have focused primarily on iron, zinc, and selenium, which are dietary deficiencies common among women, infants, and children in some developing countries. Iron deficiency is the most common nutrient deficiency in both developed and developing countries.
Table 2. Effects of Selected Nutrients on Immune Response

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Effects</th>
</tr>
</thead>
</table>
| Vitamin A   | • Improves gut barrier function  
              • Maintains production of mucosal secretions  
              • Protects against oxidative damage (in β-carotene form)  
              • Improves immune response to antigens  

  **Deficiency:** increased morbidity and mortality, increased severity of infections, reduced number of lymphocytes, reduced lymphoid organ weight |
| Vitamin C   | • Protects against oxidative damage  

  **Deficiency:** decreased resistance to infection and cancer; decreased delayed-type hypersensitivity response; impaired wound healing |
| Vitamin B₆  | • Required for nucleic acid and protein synthesis (with implications for rapid immune cell response to antigens)  

  **Deficiency:** lymphocytopenia; reduced lymphoid tissue weight; reduced responses to mitogens; general deficiencies in cell-mediated immunity; lowered antibody responses |
| Vitamin B₁₂*| • Required for nucleic acid and protein synthesis  
              • Mediates a variety of immune responses, including cell-mediated and humoral immunity  

  **Deficiency:** depressed immune responses, including delayed-type hypersensitivity response and T-cell proliferation |
| Vitamin E   | • Acts as a strong antioxidant, reduces cell membrane peroxidation  

  **Deficiency:** rare in humans (except as secondary to fat malabsorption); reduced immune response, anemia, fetal resorption in experimentally induced deficiency |
Table 2. Effects of Selected Nutrients on Immune Response (continued)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Effects</th>
</tr>
</thead>
</table>
| **Folate*** | • Required for nucleic acid and protein synthesis  
• Mediates a variety of immune responses, including cell-mediated and humoral immunity  
Deficiency: depressed immune responses, including delayed-type hypersensitivity response and T-cell proliferation |
| **Iron** | • Is fundamental for normal immune system development  
• Allows proper functioning of enzymes involved in nucleic acid synthesis and cell replication  
• Mediates components of inflammatory response  
Deficiency: reduced capacity for an adequate immune response, as measured by: decreased delayed-type hypersensitivity response, mitogen responsiveness, and NK cell activity; decreased lymphocyte bactericidal activity; lower IL-6 levels |
| **Selenium** | • Allows proper functioning of enzymes involved in drug/chemical metabolism and other processes  
• Acts as an antioxidant; protects cells from oxidative damage  
Deficiency: suppression of immune function; increased cancer incidence and cardiomyopathy in populations with chronic Se deficiency |
| **Zinc** | • Allows proper functioning of enzymes involved in nucleic acid synthesis and cell replication  
• Improves intestinal barrier function  
• Mediates unspecific immunity, such as neutrophils and NK cells  
• Has a role in balance of T helper cell functions  
Deficiency: increased susceptibility to infectious diarrhea, increased diarrheal and respiratory morbidity |

*Note: Immune system effects of vitamin B12 deficiency and folate deficiency are clinically indistinguishable.*

References 86, 88, 94-96.
Long-chain fatty acids, particularly DHA and ARA, are preferentially incorporated into cell membrane phospholipids. LCPUFA in cell membranes contribute to variations in membrane fluidity, influence on intracellular signal transduction and gene expression, and provide the substrate for eicosanoid production. Although all three effects could contribute to the immune response of many cell types, only effects related to eicosanoid production will be discussed herein.

Precursors of eicosanoids and docosanoids

Chemical mediators derived from LCPUFA include eicosanoids (a family of mediators which include prostaglandins (PG), leukotrienes, and thromboxanes), and docosanoids, as well as resolvins, docosatrienes, and neuroprotectins. These mediators act in a manner similar to hormones, but unlike hormones they have localized actions and are cleared rapidly from circulation. The production of LCPUFA-derived mediators is directed by cytokines (and other stimuli), and in turn the production of cytokines is directed by LCPUFA-derived mediators, forming complex feedback and feed-forward interactions. The overall physiological effects of eicosanoids depend on the organ or tissue of origin, the balance among individual eicosanoid types (eg, prostaglandins vs leukotrienes), and the balance among classes of eicosanoids (eg, 2-series prostaglandins derived from ARA vs 3-series prostaglandins derived from EPA).

ARA is the precursor of the 2-series prostaglandins (eg, PGE2) and thromboxanes and 4-series leukotrienes (Figure 2). These compounds are predominantly pro-inflammatory, and they also influence T-cell, B-cell, and natural killer cell activities. The actions of eicosanoids derived from dihomo-γ-linolenic acid (20:3n-6; the precursor of ARA) tend to oppose those of eicosanoids derived from ARA.

Although ARA is associated with pro-inflammatory responses, in vitro and animal studies suggest that ARA has an essential early role in immune cell growth in the thymus, and in differentiation, migration, and proliferation of immune cells. There is a substantial accretion of ARA in the mouse thymus during early growth and development, which is consistent with other findings of placental ARA enrichment in the fetus and the presence of ARA in breast milk. The pro-inflammatory effects associated with compounds derived from ARA have key roles in maintaining the health of the individual.
DHA is readily incorporated into cell membrane phospholipids, where it exerts effects on fluidity and upon the function of membrane proteins such as receptors. DHA can also be retroconverted to eicosapentaenoic acid (EPA; 20:5n-3); EPA is a substrate for synthesis of eicosanoids such as the 3-series prostaglandins (e.g., PGE3) and thromboxanes, as well as the 5-series leukotrienes (Figure 3). These have a different structure from the eicosanoids produced from ARA, which affects their potency. In general, eicosanoids derived from EPA are less inflammatory in nature than those produced from ARA.22

EPA also gives rise to resolvins of the E-series while DHA gives rise to D-series resolvins, docosatrienes, and neuroprotectins.23 These mediators have strong anti-inflammatory effects.23,68

In addition to giving rise to mediators of different activities and potencies, EPA and DHA compete with ARA for metabolism. By doing so, n-3 LCPUFA slow the production of pro-inflammatory eicosanoids derived from ARA.4,22
Early studies of LCPUFA effect on immune function

Much of the evidence for the immunomodulatory role of LCPUFA has been derived from cell culture and animal studies. These studies have shown that LCPUFA influence several areas of the immune response, including T-cell activity and proliferation, as well as cytokine secretion and antigen presentation.99 These studies suggest that changes in the fatty acid composition of immune cells and in the mediators they produce as a result of preformed LCPUFA intake (particularly n-3 LCPUFA intake) may result in altered immune responses and could potentially lead to significant effects in promoting health.

Studies in adults and children suggest relationships between intake of specific LCPUFA and changes in markers of immune function. Results have shown that:

- **Persons with allergy may have altered levels and ratios of n-3 and n-6 fatty acids, including DHA and ARA, compared with non-allergic persons.** For example, several studies in infants and children have observed inverse relationships between risk or subsequent development of allergy and atopic disease and blood levels of PUFA, including ARA.25-27,100

- **Supplementing the diet with preformed LCPUFA alters the levels of those LCPUFA in plasma phospholipids and immune cells.**4,24 LCPUFA content in infant cell membrane phospholipids is altered by dietary supplementation in both pregnancy28 and the postnatal period.29
Supplementing the diet with preformed LCPUFA results in discrete changes in immune cell and cytokine production; these changes differ with DHA vs ARA supplementation and are influenced by the ratio of n-6 to n-3 fatty acids in the diet. For example, Kelley et al. observed decreased production of PGE_2 and LTB_4 (a leukotriene), decreased natural killer (NK) cell activity, and decreased secretion of IL-1β and TNF-α cytokines in response to immunologic challenge to peripheral blood mononuclear cells isolated from healthy men supplemented with 6 g DHA/day for 90 days. For example, Kelley et al. observed decreased production of PGE_2 and LTB_4 (a leukotriene), decreased natural killer (NK) cell activity, and decreased secretion of IL-1β and TNF-α cytokines in response to immunologic challenge to peripheral blood mononuclear cells isolated from healthy men supplemented with 6 g DHA/day for 90 days. B- and T-cell functions were not affected. In another study, men were supplemented with 1.5 g ARA/day for 50 days and no changes were observed in delayed-type hypersensitivity reaction, NK cell activity, lymphocyte proliferation, or secretion of IL-2 and TNF-α. However, significant increases were observed in numbers of circulating neutrophils and in production of PGE_2 and LTB_4. These differing effects of DHA vs ARA suggest that dietary alterations to increase n-3 LCPUFA intake may have effects on markers of immune function. Furthermore, as evidenced by the different responses observed with very large dosing in adults, additional studies with varying doses in neonates and children may be necessary to fully appreciate the potential benefit of these compounds.
There has been a worldwide increase in allergic disease,\textsuperscript{103} with as many as 40\% of children in industrialized countries developing allergic sensitization. Many of these children develop associated diseases. Recent Australian figures estimate that approximately 20\% of school-aged children experienced wheezing in the previous 12 months, and that prevalence of eczema and rhinitis were roughly 17\% and 13\%, respectively.\textsuperscript{104} This allergy “epidemic” has lead to intense interest in environmental factors that may either account for this change, and/or play a role in reversing this trend. There has been a good case for exploring the role of n-3 PUFAs based on their declining levels of dietary intake and their anti-inflammatory properties. However, while a growing body of evidence suggests associations between dietary LCPUFA intake early in life and immunological outcomes including atopy, results have not been conclusive, and new studies have challenged this notion.\textsuperscript{35}

Development of atopic disease has a strong familial component; as a result, many research studies have focused on interventions in infants and children with an increased risk of atopy. Children born into atopic families have a 50\%–80\% risk of developing allergic diseases; children from families with no history of atopy have approximately a 20\% risk.\textsuperscript{105} Risk of allergy appears to be higher if both parents are allergic (vs only one parent) and if the mother (vs the father) has allergic disease (reviewed by Prescott & Tang, 2004)\textsuperscript{105}

Because maternal diet is the most important determinant of LCPUFA accretion for the woman and her infant, dietary investigations have focused on the relationship between LCPUFA intake and status in pregnant and breastfeeding women and the incidence of atopic diseases in their children. Studies have examined the effects of oily fish intake, fish oil supplementation, and supplementation with oils high in n-3 LCPUFA. These studies have evaluated interventions during three different periods of development:

- The effects of maternal LCPUFA intake during pregnancy on neonatal indicators of immune response and atopic risk,
- The effects of maternal LCPUFA intake during lactation on breast milk LCPUFA content and correlations with immune function and atopic disease in infants, and
- The relationship between LCPUFA intake during infancy and childhood and subsequent development or progression of atopic disease, particularly that affecting the respiratory system.
Effects of LCPUFA intake in pregnancy

The effect of LCPUFA intake in pregnancy on infants’ risk of developing allergy is emerging as an area of clinical interest. As with all other systems, the immune system develops in utero, and there is accumulating evidence that newborns who subsequently develop allergic disease have a number of alterations in immune function at birth (reviewed in Prescott, 2003). Together with the rising rates of allergic disease, this association suggests that environmental factors may affect neonatal “allergy risk” during pregnancy. Dietary factors, with their recognized potential to influence immune functions, are among the environmental factors thought likely to have such effect. Accordingly, several studies have noted differences in cord blood LCPUFA profiles in neonates at high risk of allergic disease compared with low-risk newborns, although the findings are not consistent. Others have noted that neonates at high risk of atopy already had differences in in vitro T-cell responses at birth. Despite speculation about biochemical alterations or metabolic abnormalities which could contribute to the development of atopy, specific relationships have not been confirmed.

More recently, Dunstan and Prescott proposed that supplementation of the maternal diet with n-3 LCPUFA during pregnancy may influence the development of allergic and other immune-mediated diseases. This proposal was based on their observations that fish oil supplementation in pregnancy can modify immune responses. In a randomized, controlled trial, allergic women (n=83) consumed 4 g fish oil/day (containing 3.7 g of n-3 LCPUFA; 56% as DHA and 27.7% as EPA) or placebo from 20 weeks gestation to delivery. Infants of LCPUFA-supplemented women had significantly higher levels of n-3 fatty acids in red blood cell membranes than infants of unsupplemented women. All neonatal cytokine responses to allergens tended to be lower in infants of supplemented women, as evidenced by a trend to lower levels of IL-5, IL-13, IL-10, and IFN-γ produced compared to those for infants of women in the control group. Cord blood IL-13 levels were significantly lower (by 65%) in the supplemented group compared to the control group; there was also a significant inverse relationship between n-3 PUFA levels in neonatal red blood cell membranes and plasma IL-13. Although the trial was not designed to evaluate clinical outcomes, infants of supplemented mothers were three times less likely than control infants to develop a positive skin prick test to egg and had significantly less severe atopic dermatitis at 1 year of age. Based on data from the same study, Barden et al. suggested that DHA may affect atopic disease by attenuating neonatal lipid peroxidation, as cord blood and urinary measures of lipid peroxidation were significantly decreased in the infants of supplemented women. The authors suggest that the findings of these studies support a role of maternal dietary LCPUFA content in influencing the infant’s immune system, which in turn may affect development of atopy. Larger studies to assess the clinical effects of early fish oil supplementation on allergy outcomes are currently underway.
Other groups have shown relationships between LCPUFA levels and neonatal cytokine responses. In a US birth cohort study, higher cord blood levels of EPA and ARA were associated with reduced cord blood lymphocyte proliferation responses and reduced production of some cytokines.\textsuperscript{110}

In contrast, Newson \textit{et al.} found no association between fetal exposure to n-3 and n-6 fatty acids, measured in cord blood erythrocytes, and development of wheezing and atopic disease at 30 and 42 months of age.\textsuperscript{111} Positive associations were noted among cord blood ARA:EPA ratio and eczema as well as linoleic acid:alpha-linolenic acid ratio and later-onset wheeze, but these associations did not persist when data were adjusted for multiple comparisons. Women enrolled in this longitudinal study were not supplemented with any particular form of fatty acids. The authors assert that, because of the interaction between maternal supply, fetal demand, and transfer kinetics, cord blood fatty acid levels of LCPUFA are more likely to reflect fetal exposure in late gestation than maternal blood levels.\textsuperscript{111} As mentioned previously, dosing may play a significant role in these apparently disparate effects.

\section*{Effects of maternal LCPUFA status during lactation}

As a component of studies described previously, Dunstan and coworkers looked at the effects of maternal fish oil supplementation on breast milk LCPUFA as well as markers of mucosal immunity (IgA, sCD14) and cytokines (IL-5, IL-6, IL-10, TNF-\alpha, and IFN-\gamma) in breast milk.\textsuperscript{21} At 3 days postpartum, breast milk DHA and EPA levels were significantly higher among supplemented women compared to control women, and milk ARA levels were significantly lower. Milk DHA levels were positively correlated with milk IgA content. Furthermore, cytokines involved in IgA synthesis (IL-10 and IL-6) were significantly correlated with milk levels of IgA and n-3 PUFA, although no differences were noted among study groups in breast milk levels of IgA, sCD14, or cytokines. These results suggest a potential effect of n-3 LCPUFA on mucosal immune development measured in the early postnatal period.

Numerous studies have provided evidence of relationships between breast milk LCPUFA composition and incidence or markers of atopic disease in infants and children.\textsuperscript{112-117} In particular, studies have shown that low levels of n-3 fatty acids in breast milk are associated with increased incidence of atopic disease and other allergic manifestations.\textsuperscript{118} Milk from mothers of allergic children was lower in total n-3 LCPUFA and had a higher n-6 to n-3 PUFA ratio compared to mothers of non-allergic children.\textsuperscript{112,113} Further, low concentrations of n-3 LCPUFA and a high ratio of ARA:EPA in breast milk were related to symptoms of allergy at 18 months of age.

These effects are thought to be mediated by the influence of LCPUFA on maternal cytokines in breast milk, although clinical studies have not yielded definitive conclusions. Hawkes \textit{et al.} reported that supplementation of lactating women with 300 or 600 mg DHA for 4 weeks resulted in elevated DHA levels in breast milk, plasma, and selected immune cells, but did not
yield differences in cytokine levels. The authors suggested that large variability contributed to the inadequate power to detect differences, should they exist. In another study, in vitro production of IFN-γ in whole blood cultures from infants whose mothers received 1.5 g/day of n-3 fatty acids from fish oil postnatally for 4 months had a median IFN-γ level four times higher than that of control infants when measured at 2 ½ years of age; IL-10 levels were similar. The authors speculated that fish oil supplementation during lactation may have caused a shift in infants’ immune polarization and more rapid maturation of their immune systems, resulting in a long-term immunomodulating effect. A mechanism for such an effect was not defined.

Several studies have shown a relationship between maternal LCPUFA intake and development of atopic dermatitis in their breastfed infants and children. A study of women with atopic disease (ie, asthma, allergic rhinitis, atopic dermatitis) and their breastfed infants found that infants who developed dermatitis (38% of the sample total) had consumed breast milk with a higher ratio of saturated to polyunsaturated fatty acids. Notably, breast milk stearic acid levels were increased and total n-3 fatty acids and EPA were decreased compared to milk consumed by infants who did not develop atopic disease.

Oddy et al. examined the relationship between breast milk fatty acids and the later development of atopy and eczema in high-risk children who were breastfed for at least 6 months. They reported significant associations between non-atopic eczema at 6 months and an increased ratio of n-6 to n-3 fatty acids in breast milk samples collected at 6 weeks and 6 months of lactation. The authors concluded that milk fatty acids significantly modulated non-atopic eczema in infants at 6 months.

Wijga et al. observed that risk of allergic symptoms in susceptible infants and children was inversely associated with breast milk n-3 LCPUFA content. In a study of 265 mothers (158 allergic and 107 nonallergic) and their children, inverse associations were observed among children of mothers with allergy but not among children of mothers without allergy. Milk levels of total n-3 LCPUFA and ratios of n-3 to n-6 LCPUFA were inversely associated with eczema at 1 year among children of allergic mothers.

Kankaanpaa et al. found that the milk of atopic vs nonatopic women (n=20 each group) differed slightly in n-3 fatty acid content. Milk from atopic women also contained less γ-linolenic acid (an n-6 LCPUFA) than milk from nonatopic women. Similarly, atopic infants had lower serum levels of γ-linolenic acid than healthy infants (n=10 each group). No differences were found in maternal PUFA intake. The authors suggested that risk of atopic disease may be increased by high intake of dietary n-6 LCPUFA or low intake of γ-linolenic acid and n-3 LCPUFA.

These relationships are equivocal, however. Stoney et al. found a positive association between breast milk (ie, colostrum) n-3 fatty acid levels and allergic sensitivity. In particular, they found that infants testing positive to skin prick tests of common allergens had been exposed to colostrum containing higher levels of n-3 fatty acids, eg, DHA and docosapentaenoic acid (DPA). The study evaluated 224 mother-infant pairs from families in
which at least one first-degree relative had an atopic disease. Stoney et al. concluded that higher n-3 fatty acids in colostrum could be a risk factor for atopy in high-risk breastfed infants. They suggested that the apparent contrast between their study findings and those of other investigators could be explained by differences in subjects’ risk of allergy, study analysis methods, age at which sensitization was measured, and definition of atopy used.

Associations were noted in another study evaluating the relationship between colostrum fatty acid levels and later development of allergy. At 1 year of age, high linoleic acid levels were associated with high IgE response to cow’s milk protein, and low levels of DPA were associated with elevated total IgE, but no associations were observed between colostrum fatty acids and atopic eczema.

Although many questions remain to be answered, these studies and others provide preliminary evidence that exposure to n-3 LCPUFA in utero and via breast milk may be associated with reduced development of atopic disease in infants and children. This association now needs to be confirmed. If atopic disease is in fact correlated with intake of n-3 PUFA, then it is reasonable to consider that dietary interventions which result in altered amounts and ratios of n-6 to n-3 fatty acids may be associated with modulation of atopic disease. Such findings could have important implications for public health policy and nutrient intake recommendations, but more studies are needed.

Effects of infant formula supplementation

Whether similar effects extend to infants fed LCPUFA-supplemented formula remains to be determined. In a study with preterm infants, Field et al. evaluated the effect on immune system measures of feeding supplemental DHA and ARA in preterm formula from about day 8 to day 42. Supplemented infants had lymphocyte populations, phospholipid LCPUFA composition, cytokine production, and antigen maturity more like that of a breastfed reference group and less like that of a non-supplemented control group. Similar results were observed in a study with term infants: infants fed supplemented formula had lymphocyte populations and immune cell maturity more consistent with the breastfed group. Although results from these studies imply that providing supplementary DHA and ARA in infant formula may enhance infants’ ability to respond to immune system challenges, the clinical significance is unknown.

A recent nonblinded, nonrandomized clinical survey (n=1342) reported that term infants who were fed an LCPUFA-supplemented formula (17 mg DHA/100Cal and 34 mg ARA/100 Cal) for the first year of life had improved respiratory health compared to control infants. This survey was conducted with 357 pediatricians and was not blinded or randomized. Infants in the control group were fed formulas commercially available in Europe, including unsupplemented formulas and a few formulas supplemented with DHA and ARA at levels that were lower than the LCPUFA-supplemented formula described
above. Whether formulas supplemented with lower levels or different sources of DHA or ARA would show similar improvement was not tested. Nonetheless, because of the results observed in this very large clinical survey, a large, randomized trial of LCPUFA supplementation during infancy would be of interest.

Relationship between LCPUFA intake in infancy and childhood and subsequent health outcomes

The relationships between infant and childhood intake of LCPUFA and subsequent health outcomes have been of great interest for many years. Although interest was initially centered around effects on neurodevelopment, it has more recently expanded to include other outcomes, including potential benefits for immune development and allergy risk. A number of studies have explored the relationship between dietary LCPUFA in infancy and the development of allergic sensitization and associated conditions, including asthma and allergic airway inflammation and allergic rhinitis.

Asthma, a chronic inflammatory disease of the respiratory tract, is a common condition in childhood and is commonly associated with atopy; its prevalence has been increasing worldwide in both children and adults for several decades.103 This rising prevalence has raised significant health concern. Early population studies suggested that a decreased risk of asthma may be associated with consumption of fish, a dietary source of n-3 fatty acids.5,125 Subsequent birth cohort studies have also reported that the ratio of n-6 to n-3 LCPUFA may influence the development of asthma.126

To date, there has only been one published intervention study to examine the effects of fish oil supplementation in early childhood on the prevention of asthma and allergy.35-37 The Childhood Asthma Prevention Study (CAPS) was a randomized, controlled trial which separately assessed the preventive effects of house dust mite avoidance and increasing consumption of n-3 fatty acids. The study investigators recruited pregnant women (n=616) whose unborn children were deemed to be at risk of developing asthma. Women randomly assigned to the dietary intervention group received a daily supplement of tuna fish oil (500 mg oil/day) to give their children along with margarines and cooking oils rich in n-3 fatty acids to use in food preparation; women in the control group received a placebo supplement for their children and polyunsaturated margarines and cooking oils. The children were assessed clinically at 18 months, 3 years, and 5 years of age.

Initial findings were encouraging, with reduced respiratory symptoms such as wheeze at 18 months of age and reduced coughing (among atopic infants) in the “active” diet group at 3 years of age.36,37 However, there was no reduction in the development of asthma. At 5 years of age, there was no reduction in the prevalence of asthma, wheezing, atopic dermatitis, or allergic sensitization in children who had received n-3 PUFA enriched diets36 despite confirmed effects on LCPUFA status during the intervention.36 A number of other studies
are on-going which will assess the effects of supplementation with higher doses of n-3 PUFA from birth.

Omega-3 fatty acids have also been investigated in the management of established asthma in both children and adults. A recent Cochrane systematic review of nine randomized, controlled studies compared n-3 PUFA supplementation with placebo or with n-6 PUFA supplementation and found no evidence to support the use of marine n-3 PUFA in the management of asthma.127 Seven of the studies were conducted in adults and two in children. Of the two studies conducted in children, one reported that fish oil supplementation was associated with improved asthma symptom scores and reduced medication usage.128 Hodge et al.129 observed no improvement in asthma severity in children supplemented with n-3 PUFA rich fish oil. However, a significant improvement in peak expiratory flow and asthma medication use following fish oil supplementation was described when these results, as well as additional data provided by Hodge and colleagues, were evaluated in the Cochrane analyses.127 While inconclusive, these observations highlight the need for further studies in this area, particularly in children with asthma.

Thus, it still may be too early to draw definitive conclusions about the influence of n-3 fatty acids on asthma, despite increasing positive evidence of their effect. At the request of the Office of Dietary Supplements, US National Institutes of Health, Schachter et al. evaluated the published literature on the topic of n-3 fatty acid supplementation and asthma.130 They concluded that there are not enough data from well-designed studies with which to evaluate the influence of n-3 fatty acids on mediators of inflammation thought to be involved in asthma, or the actual role of those mediators in asthma. Results from additional carefully designed studies will be useful.
It has been suggested that fatty acids act as “gatekeepers” of immune cell regulation, with direct effects on the activities of cells and mediators involved in immune system response. Emerging data from clinical and animal studies suggest that dietary n-3 fatty acids provided in appropriate ratios with n-6 LCPUFA and as part of a balanced diet in early life (beginning in utero) may influence immune system development, immune cell function, and incidence of atopic disease. Correlations between maternal intake of n-3 LCPUFA and incidence of atopic disease in infants suggest that there may be a critical time period during which early exposure to n-3 and n-6 dietary fatty acids may influence immune system development and function. Development of atopy in the infant and child appears to be influenced by maternal intake and serum levels of LCPUFA as well as the ratio of dietary n-6 to n-3 fatty acids during pregnancy. These findings suggest that prenatal fatty acid supply is more important to infant immune development than previously understood.

Because n-3 LCPUFA (eg, EPA and DHA) support anti-inflammatory and immune responses, dietary modification with these nutrients may have a potential role in influencing the development of allergic disease, including inflammation, in early life. Results of clinical studies in pregnant and lactating women, infants, and children suggest that interventions which increase intake of these fatty acids may have significant clinical effects on incidence and manifestations of atopy.

Although some n-6 LCPUFA are associated with pro-inflammatory immune responses, emerging evidence in animal and human studies suggests that n-6 LCPUFA, including ARA, have important roles in immune system development and regulation. Providing preformed DHA and ARA in infant formula at levels close to those in human milk may help formula-fed infants compensate for a potential immunological disadvantage compared to breastfed infants. Supplemented formulas have been shown to be safe for term and preterm infants when both DHA and ARA are added to the formula. The optimal levels of n-3 LCPUFA remain to be determined; it is prudent in the meantime to follow worldwide average levels in human milk, although it is interesting to consider the potential benefit of higher levels of supplementation. Nonetheless, despite evidence of benefit for some LCPUFA-supplemented formulas, the clinical effect of preformed LCPUFA in the infant diet remains controversial.
Although much insight has been gained in the past decade about how the immune system develops and how dietary factors affect that development, numerous questions remain. In particular, additional research should consider:

- The effects of study design on trial outcome (such as sample size, immune parameters evaluated, methods of measurement, sources and levels of LCPUFA supplementation, duration of intervention, and age at initiation of supplementation);
- The effects of confounding factors on trial outcome and reproducibility (including hereditary risk, sampling methods, regional dietary differences, and nutrient-nutrient and nutrient-gene interactions);
- The extent to which results from cell culture and animal studies can be replicated by clinical experiments;
- Potential mechanisms by which LCPUFA can modulate clinical outcomes; and
- The differing effects of ARA, EPA, and DHA on immune responses.

Despite unanswered questions, clinical evidence to date suggests that relatively simple dietary interventions may influence the incidence and severity of allergic diseases among infants and children worldwide. The particular characteristics of the most effective interventions remain to be determined.


---

**References**


