Abstract
There has been growing interest in the role of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in the modulation of the immune response during early childhood and whether this may translate to a reduction in childhood allergic disease. Several randomized controlled trials of n-3 LC-PUFA supplementation have been reported, largely involving children who are at high hereditary risk of developing allergies. These studies relatively consistently indicate that supplementation during pregnancy results in fewer children with atopic eczema in early childhood. On the other hand, supplementation studies confined exclusively to the postnatal period have demonstrated mixed results with one trial showing no effect and the other suggesting a transient effect on symptoms of respiratory disease. In summary, supplementation with n-3 LC-PUFA during the perinatal period and before allergic response is established may be a useful strategy to prevent early childhood allergic disease in children at high hereditary risk. Further work is needed to establish the optimal period of supplementation and whether longer term benefits exist.

Burden of Allergic Disease in Childhood
The incidence of allergic diseases in Australia and other industrialized countries has increased over the last 20 years and is now estimated to be at least 20% [1–3]. Common allergies include allergic rhinitis or hay fever, asthma, eczema or atopic dermatitis and food allergies. The risk of allergic disease is increased to about 1 in 3 if one first-degree relative (parent or sibling) is atopic and to 70% if both
The pattern of allergy expression differs with the age of the child, with the greatest incidence of food allergy and atopic eczema peaking by 1 year of age while asthma and allergic rhinitis continue to rise until around 15 years of age [5]. Regardless of this changing pattern through childhood, many childhood allergies persist with about 50% of childhood asthma sufferers and 80% of hay fever sufferers continuing to have symptoms into adulthood [6, 7]. The cost to the health care system and the burden to the family are high [8, 9]. The estimated annual cost is approximately $9.4 billion in Australia and $20 billion in the United States of America [10].

**Why Are Rates of Allergic Disease So High?**

There is strong evidence that environmental factors commensurate with higher socioeconomic conditions and hygiene standards have contributed to the increased prevalence of allergic disease across the western world. Societies with fewer respiratory infections, greater use of antibiotics early in life, fewer older siblings in the household, less contact with farm animals and general lack of early microbial exposure are repeatedly associated with the greatest burden of allergic disease [11, 12]. The atopic predisposition is believed to arise where the individual has an innate tendency to produce IgE antibodies (sensitization) which in some individuals progresses to allergic disease. The allergens causing sensitization are nearly always proteins originating from environment pollens, house dust mite or food. Just why modern day societies are becoming increasingly sensitized to environmental allergens is a matter of debate. Several factors are thought to be involved including whether the child is breastfed from birth [13], the antioxidant status of the individual [14] and the balance of n-6:n-3 polyunsaturated fatty acids (PUFA) in the diet [15].

**Dietary n-3 LC-PUFA Intervention – A Chance to Modulate Allergic Disease?**

Over the last 30 years, experimental studies have described the importance of marine oils, rich in n-3 LC-PUFA, in altering cell membrane phospholipid fatty acid profiles and modulating a range of inflammatory events [16–18]. Current Western diets favor the intake of n-6 fatty acids via increased consumption of linoleic acid (18:2n-6)-rich vegetable oils and increased consumption of meat-based food items, leading to a predominance of arachidonic acid (AA, 20:4n-6) in tissues [19]. AA gives rise to eicosanoids such as prostaglandin E₂ that can
enhance the synthesis of T helper 2 type cytokines (IL-4, IL-5, IL-13) and IgE antibodies – the hallmark of atopic responses to environmental allergens [20]. When diets are high in n-3 LC-PUFA (e.g. fish), they are readily incorporated into cellular phospholipids, in the process displacing AA. This leads to a range of biochemical changes and immunologic effects, including reduction of prostaglandin E2 synthesis, alteration of receptor expression and activity and reduced responses to cytokines known to be associated with allergies [18, 21]. Thus, there are plausible mechanisms by which diets high in n-3 LC-PUFA may modulate the development of allergic disease and regulate immune responses. Furthermore, the substantial shift in dietary fatty acids to favor n-6 over n-3 fatty acids has coincided with the increased prevalence of childhood allergic disease, leading to speculation that the two may be linked [15, 22, 23].

**Epidemiological Studies Investigating the Associations between n-3 LC-PUFA Intake and Childhood Allergies**

While there is limited evidence that n-3 LC-PUFA have a major therapeutic role in established allergic disease [24, 25], there is growing interest and support for an association between n-3 LC-PUFA intake during early life and allergy prevention in children. In fact, the last 5 years have witnessed the publication of 7 large cohort studies involving a total of 1,534 mother-child pairs that have assessed the association between fish intake in pregnancy, the richest source of n-3 LC-PUFA, and allergic disease outcomes in children. Five of these 7 studies have reported that higher fish intake during pregnancy is associated with fewer symptoms of allergic disease in early childhood [26–30]. The 2 studies that showed no association between fish intake in pregnancy and allergic disease outcomes in children were both conducted in Japan, where the baseline fish intake is higher than most other parts of the world. Interestingly, the only 2 studies to extend their follow-up to 6 years of age reported that high fish intake (at least 2 fish meals per week) during pregnancy was associated with a reduction in atopic wheeze [28] and a reduction in persistent wheeze [27]. Although the apparent consistency of these associations is promising, it is not possible to infer a causal link between increased n-3 LC-PUFA exposure (via fish) in pregnancy and the reduction of allergic disease in children because it is not possible to exclude the presence of residual confounding from environmental factors or that there may be constituents of fish other than n-3 LC-PUFA driving these associations. It is for this reason that evidence from randomized controlled trials (RCT) is essential to establish whether increasing the intake of n-3 LC-PUFA during early life will alter the pattern of allergy development in childhood.
Evidence from RCTs: n-3 LC-PUFA Intervention during Early Life and Childhood Allergies

The available RCTs can be considered according to whether n-3 LC-PUFA supplementation was commenced during the prenatal or postnatal period. Should any interventions be effective, this distinction is important for understanding the optimal timing of exposure.

n-3 LC-PUFA Supplementation during the Prenatal Period

The largest RCT, specifically designed to assess allergy outcomes, is the nested allergy follow-up of the DOMInO (DHA to Optimise Mother and Infant Outcome) Trial [31, 32]. Women with singleton pregnancies were randomly assigned to receive identical looking capsules containing either fish oil concentrate (900 mg n-3 LC-PUFA/day) or a blend of vegetable oils (no n-3 LC-PUFA) from about 20 weeks gestation until birth. Following randomization into the DOMInO trial, women were asked a series of structured questions to determine the allergy risk of their unborn fetus. Families were eligible for the allergy follow-up if the mother, father or sibling of the unborn child had a medical diagnosis of asthma, allergic rhinitis, or eczema as the risk of allergic disease is increased to about 1 in 3 if one first-degree relative (parent or sibling) is atopic [4]. Informed consent for the nested allergy follow-up was sought during pregnancy once eligibility was confirmed. Of the 1,080 eligible families, 706 consented and were included in the allergy follow-up. At one year of age, fewer infants who were exposed to n-3 LC-PUFA supplementation in utero were diagnosed with atopic eczema compared with control infants (26/368, 7.1% DHA vs. 39/338, 11.7% control; unadjusted relative risk, 0.61; 95% CI: 0.38–0.98; p = 0.04; adjusted relative risk, 0.64; 95% CI: 0.40–1.02; p = 0.06), although the percentage of infants diagnosed with any IgE-mediated allergic disease (eczema and/or food allergy with sensitization) did not reach statistical significance (32/368, 8.6 vs. 43/338, 12.7%; unadjusted relative risk, 0.68; 95% CI: 0.43–1.05; p = 0.08; adjusted relative risk 0.70; 95% CI: 0.45–1.09; p = 0.12) [32]. Interestingly, fewer infants in the n-3 LC-PUFA group were sensitized to egg compared with control (34/368, 9.3% n-3 LC-PUFA vs. 52/338, 15.4% control; unadjusted relative risk, 0.61; 95% CI: 0.40–0.91; p = 0.02; adjusted relative risk, 0.62; 95% CI: 0.41–0.93; p = 0.02) but relatively few infants had medically diagnosed food allergies, and the rates did not significantly differ between the groups probably because it was not possible to unequivocally confirm egg allergy diagnosis in a proportion of infants who had suspected reactions as infants were refusing to eat egg [32].
These results are corroborated by 4 other recent n-3 LC-PUFA intervention studies during pregnancy, although these other studies were either not specifically designed or powered to assess clinical outcomes. Dunstan et al. [33] and Krauss et al. [34] both demonstrated downregulation of cord blood mononuclear cell cytokine responses (IL-5, IL-13) to allergens in response to fish oil treatment during pregnancy compared with placebo, as well as upregulation of TGF-β in cord blood [34]. Collectively, these data indicate a modulation of the neonatal immune response towards a less allergic phenotype in response to n-3 LC-PUFA supplementation. These data are further supported by a recent Swedish study reporting fewer children with IgE-associated eczema at 1 and 2 years age in response to n-3 LC-PUFA supplementation from the 25th week of gestation until end of lactation (1 year: 4/52, 8% vs. 15/63, 24%, p < 0.05 [35]; 2 years: 5/54, 9% vs. 15/63, 24%, p < 0.04 [36]) and are consistent with the trends observed by Dunstan et al. [33] in which infants of atopic women treated with fish oil were generally less likely to develop positive skin prick tests (IgE-mediated atopic response) or have allergic symptoms compared with controls. Although the small numbers in these latter 2 trials cannot exclude the possibility of random error, they are consistent with the results of the single largest trial which suggests that about 1 g of n-3 LC-PUFA during the last half of pregnancy reduces the risk of atopic eczema in infants at high hereditary risk of allergic disease [32]. Clearly, data from longer term follow-up studies will be important to verify whether these effects are long lasting.

The only relevant long-term follow-up came from Olsen et al. [37] who reported a registry-based 16-year follow-up of adolescents whose mothers were allocated to fish oil (n = 263), olive oil (n = 136) or no treatment (n = 136) during the last 10 weeks of pregnancy. Although the rate of asthma in the fish oil group (8/263, 3.3%) was lower than the olive oil group (11/136, 8%), the frequency in the no treatment group was also low (3/129, 2.3%). These surprisingly low percentages across all groups and the lack of consistently controlled outcome assessments necessitate further research.

**n-3 LC-PUFA Supplementation during the Postnatal Period**

Two relatively large trials have been specifically designed to assess the effect on n-3 LC-PUFA supplementation during the postnatal period [38, 39]. One trial began intervention during the newborn period [38], while the other started at 6 months of age once infants commenced weaning [39].

D’Vaz et al. [38] randomly allocated 420 newborn infants at high atopic risk to a daily supplement of fish oil (about 400 mg n-3 LC-PUFA per day) or to an
olive oil control until they were 6 months of age. 323 infants were assessed for allergy outcomes at 12 months. There were no group differences in the frequency of any allergic outcomes including sensitization, eczema, food allergy or asthma. Interestingly, there were lower rates of adherence in the fish oil group compared with control and significantly greater loss to follow-up and a higher percentage of withdrawals from the fish oil group compared with control, raising the possibility of a systematic bias.

The Childhood Asthma Prevention Study included 616 high-risk infants who were randomly allocated to a fish oil supplement as well as margarines and cooking oils with a high n-3:n-6 ratio – or a vegetable oil placebo as well as margarines and cooking oils with a low n-3:n-6 ratio [39]. The intervention commenced from around 6 months of age and included modification of solid foods. The n-3 fatty acid intervention group received a supplement of tuna oil and canola oil-based oils and spreads for use in cooking, while the control group received a vegetable oil supplement and n-6-rich polyunsaturated oils and spreads for use in home prepared foods. Atopy and symptoms of asthma were assessed at 18 months, 3 and 5 years of age. Although the intervention resulted in a reduction in the proportion of children with wheeze at 18 months [40] and cough at 3 years of age [41], there were no differences in the prevalence of atopy, asthma or wheeze at 5 years [42]. The implications of these transient effects are not clear, and further research is needed to determine whether prenatal n-3 LC-PUFA interventions are more effective than postnatal interventions or whether n-3 LC-PUFA interventions need to be sustained to influence allergy and asthma outcomes in the longer term.

**Implications for Research and Practice**

Supplementation during pregnancy with at least 1 g n-3 LC-PUFA per day for women carrying a fetus at high hereditary risk of allergic disease may reduce the risk of atopy and eczema in early postnatal life. The data from studies which directly supplement at risk infants with n-3 LC-PUFA are not as clear cut, although a longer term supplementation trial demonstrating some transient benefit may indicate that sustained supplementation is required [40–42]. In summary, supplementation with n-3 LC-PUFA during the perinatal period and before allergic response is established may be a useful strategy to prevent early childhood allergic disease in children at high hereditary risk. Further work is needed to establish the optimal period of supplementation and whether longer term benefits exist.
Disclosure Statement

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References


