Fish Oil Supplementation of Lactating Mothers Affects Cytokine Production in 2 1/2-Year-Old Children

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ABSTRACT: n-3 PUFA influence immune functioning and may affect the cytokine phenotype during development. To examine whether maternal fish oil supplementation during lactation could modify later immune responses in children, 122 lactating Danish mothers with a fish intake below the population median were randomized to groups supplemented for the first 4 mon of lactation with 4.5 g/d of fish oil (equivalent to 1.5 g/d of n-3 long-chain PUFA) or olive oil. Fifty-three mothers with a fish intake in the highest quartile of the population were also included. The FA composition of erythrocyte membranes was measured at 4 mon and at 2 1/2 yr. Plasma immunoglobulin E (IgE) levels and cytokine production in lipopolysaccharide-stimulated whole-blood cultures were determined at 2 1/2 yr. Erythrocyte n-3 PUFA at 4 mon were higher in infants from the fish oil group compared with the olive oil group (P < 0.001) but were no longer different at 2 1/2 yr. The median production of lipopolysaccharide-induced interferon γ (IFN-γ) in the fish oil group was fourfold higher than that in the olive oil group (P = 0.034), whereas interleukin-10 (IL-10) production was similar. The IFN-γ/IL-10 ratio was twofold higher in the fish oil group (P = 0.019) and was positively correlated with 20:5n-3/20:4n-6 in erythrocytes at 4 mon (P = 0.050). The percentages of atopic children and plasma IgE were not different in the two groups, but the study was not designed to look at atopy. Cytokine responses and erythrocyte FA composition in children of mothers with a high fish intake were intermediate in comparison with those in the randomized groups. Fish oil supplementation during lactation resulted in increased in vitro IFN-γ production in the children 2 yr after the supplementation was given, which may reflect a faster maturation of the immune system.


Because of the immunosuppressive action of n-3 PUFA, there has been some concern about the safety of an increased intake of n-3 long-chain PUFA (LCPUFA) in infancy (1). However, it has also been hypothesized that an increased intake of n-3 PUFA may protect against atopy (2). There has been an increase in the prevalence of atopic diseases in the last decades, which could be due to environmental factors. Atopic sensitization occurs early in life and may therefore be specifically sensitive to environmental factors, e.g., diet, that are introduced in this period. It is therefore possible that some “nutritional programming” of the immune system may occur.

Infants are born with an immature immune system characterized by a polarization of T helper lymphocytes (Th) toward a proallergic Th2-type response. The capacity to induce protective Th1 immune responses is impaired in early childhood, and immune maturation in childhood is characterized by a Th1 polarization. Breast milk contains numerous components that may promote the development of the infant’s immune system (3), including PUFA. The maternal diet is the most important determinant of infant PUFA accretion in membranes of breast-fed children (4). Thus, variations in maternal intake of PUFA may influence the maturation and polarization of the infant immune system.

Immune maturation occurs faster in breast-fed than in formula-fed infants and is enhanced by the addition of LCPUFA to infant formula (1,5). Fish oil (FO) supplementation of pregnant women has been shown to affect immune function in the neonate and atopic sensitization during early life (6,7). Some longitudinal studies found that a higher n-3 PUFA content in breast milk was associated with a decreased likelihood of atopy in infants (8–10), whereas another study found contrasting results (11). Supplementation with n-3 PUFA during lactation has been found to reduce the prevalence of wheezing during the first 18 mon of life (12). The effect of maternal FO supplementation during lactation on later immune function in the offspring has not been investigated.

We performed a randomized trial in which the n-3 LCPUFA intake of breast-fed infants was raised via FO supplementation to the mother during the first 4 mon of lactation. The trial was designed to investigate the effects on breast-milk FA composition, n-3 PUFA levels in infant erythrocytes (RBC), and development during the first year of life (13). The long-term effect on immune function was investigated at a follow-up visit, when the children were 2 1/2 yr old. The aim of this study was to see whether maternal FO supplementation during lactation would affect later immune function, determined by the cytokine phenotype and assessed by the in vitro production of interferon γ (IFN-γ) and interleukin 10 (IL-10) and plasma immunoglobulin E (IgE). The study did not have the power to look at atopic sensitization.

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Abbreviations: 20:5n-3, eicosapentaenoic acid (individual fatty acids are named by the number of carbon atoms:number of double bonds followed by the position of the last double bond:); FO, fish oil; HFl, high fish intake; IFN-γ, interferon γ; IL, interleukin; IgE, immunoglobulin E; LCPUFA, long-chain polyunsaturated fatty acid; LPS, lipopolysaccharide; OO, olive oil; PGE2, prostaglandin E2; RBC, erythrocytes; Th, T helper lymphocytes.
SUBJECTS AND METHODS

The study hypothesis was tested in a parallel-group randomized trial. A diagram of the trial profile, with special focus on the present follow-up study, is shown in Figure 1. The details of the study design, recruitment procedure, and subjects, which have been reported elsewhere (13), are described briefly here.

During 1999, participants were selected from among pregnant women recruited for the Danish National Birth Cohort (14) based on their intake of n-3 LCPUFA. Women with a fish intake below the population median (<0.4 g n-3 LCPUFA/d) were recruited for the randomized intervention trial, and women with a fish intake in the upper quartile (>0.8 g n-3 LC-PUFA/d) as a high-fish-intake reference group (HFI group). The inclusion criteria were: an uncomplicated pregnancy, pre-pregnancy body mass index < 30 kg/m², no metabolic disorders, and the intention to breast-feed for at least 4 mon. Furthermore, the newborns had to be healthy, term, singleton infants with normal weight for gestation (15) and an Apgar score > 7, and the mothers were to begin taking the supplements within 2 wk after birth. One hundred twenty-two and 53 of the women with a low and high fish intake, respectively, fulfilled all criteria.

The protocols for the intervention trial and follow-up study were approved by the local scientific ethical committee (KF 01-300/98 and KF 01-183/01). Both parents of all participating children gave written consent to participate after the study had been explained to them orally as well as in writing.

Trial and supplements. After birth, women with a fish intake below the median were randomly allocated to daily supplementation for the first 4 mon of lactation with microencapsulated FO or olive oil (OO) given in müesli bars (Halo Foods Ltd., Tywyn Gwynedd, Wales, United Kingdom). The FO supplement (Dry n-3TM; BASF Health and Nutrition A/S, Ballerup, Denmark) provided 1.5 g/d of n-3 LCPUFA (equivalent to 4.5 g/d of fish oil). As an alternative, the supplements were offered in homemade cookies or oil capsules (a gift from Lupe/ProNova Biocare, Lysaker, Norway). The overall self-reported compliance in both groups was, on average, 91% (range 67–100%, n = 64). Investigators and families were blinded to the randomization throughout the first year of life.

One hundred seven mothers complied with the criterion for exclusive breast-feeding for 4 mon. Mothers who did not fulfill this criterion were not excluded from the trial, but we estimated to which extent breast milk covered the energy needs of the infants from their intake of formula and complementary food. Breast milk was estimated to be the dominant source in all but 15 of the infants, most of whom were from the FO group [but the overall degree of breast-feeding did not differ between the two randomized groups (P = 0.059)]. The typical infant formulas on the Danish market at the time had an n-6/n-3 PUFA ratio of around 10 and contained no LCPUFA. One hundred mothers completed the intervention, and 50 mothers from the HFI group remained in the study for the initial 4-mon period. The biochemical effect of the intervention was assessed from the FA composition of breast milk and RBC from mothers and infants at the end of the intervention (13).

Follow-up study. When the children were 2½ yr old, all 150 families were invited to participate in the follow-up examination.