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Timing of allergy preventive and immunomodulatory dietary interventions – are prenatal, perinatal or postnatal strategies optimal?

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Abstract

The increasing allergy prevalence in affluent countries may be caused by reduced microbial stimulation and a decreased dietary $\omega$-3/$\omega$-6 long chain polyunsaturated fatty acid (LCPUFA) ratio, resulting in an abnormal postnatal immune maturation. The timing of allergy preventive probiotic and $\omega$-3 LCPUFA interventions is critical, as early life events occurring during critical windows of immune vulnerability can have long-term impact on immune development. The maternal dietary and microbial environment during pregnancy may program the immune development of the child. Prenatal environmental exposures may alter gene expression via epigenetic mechanisms, aiming to induce physiological adaptations to the anticipated postnatal environment, but potentially also increasing disease susceptibility in the offspring if exposures are mismatched. Although the importance of fetal programming mostly has been studied in cardiovascular and metabolic disease, this hypothesis is also very attractive in the context of environmentally influenced immune-mediated diseases. This review focuses on how prenatal, perinatal or postnatal $\omega$-3 LCPUFA interventions regulate childhood immune and allergy development and if synergistic effects may be obtained by simultaneous probiotic supplementation. We propose that combined pre- and postnatal preventive measures may be most efficacious. Increasing knowledge on the immunomodulatory effects of prenatal, perinatal and postnatal interventions, will help to direct future strategies to combat the allergy epidemic.

Key words: allergy, atopy, childhood, prevention, $\omega$-3 long chain polyunsaturated fatty acids, probiotics, immune regulation, cytokines, epigenetics, pregnancy
Introduction

The allergy epidemic in affluent countries must be counteracted by research identifying successful preventive measures. Supplementation of allergy protective factors that may have been lost in countries with a Western lifestyle, such as microbial stimulation with probiotics [1-8] and ω-3 long chain polyunsaturated fatty acids (LCPUFA) to redress the decreased dietary ω-3/ω-6 LCPUFA ratio [8-16], are currently evaluated in allergy intervention trials. The timing of such interventions is critical, as early life events occurring during critical windows of immune vulnerability can have long-term impact on immune development [7, 8, 17]. Factors influencing the early education and maturation of the immune system are thus especially important for subsequent allergy development [7, 8, 17, 18].

Immunomodulatory effects of pre- and perinatal supplementation with ω-3 LCPUFA

In order to develop efficacious preventive measures, it is crucial to understand the underlying immunological mechanisms and how infant immune responses are affected by the interventions. Increasing the dietary ω-3/ω-6 LCPUFA ratio may have anti-inflammatory effects [19]. Thus, the ω-6 polyunsaturated fatty acid arachidonic acid gives rise to eicosanoids including prostaglandin D₂, prostaglandin E₂ and leukotriene B₄, with significantly higher inflammatory potential than the ω-3 derived 3-series prostaglandins and 5-series leukotrienes [19]. Furthermore, a low ω-3 LCPUFA intake may be associated with decreased formation of n-3 derived resolvins and protectins, which reduce and actively resolve inflammation in animal models [20], although their anti-inflammatory potential in humans is not yet well studied [19]. Supporting the theory of infant immunomodulatory effects of ω-3 fatty acids, supplementation with ω-3 LCPUFA rich fish oil during pregnancy resulted in lower cord blood levels of the Th2 cytokines IL-4 [21] and IL-13 [21, 22]. Furthermore, we have found that fish oil supplementation during pregnancy and lactation resulted in lower ratios of the Th2-associated chemokine CCL17 to the Th1-associated chemokine CXCL11 in the circulation of infants without, but not with, maternal
history of allergy, as well as increased IgG titers to diphtheria and tetanus toxins after vaccination in nonallergic, but not allergic, infants [23]. Also, fish oil supplementation in allergic women during pregnancy was associated with a higher percentage of cord blood CD34+ hemopoietic progenitor cells and more IL-5 responsive eosinophil/basophil colony forming units [24], as well as reduced neutrophil LTB4 production in neonates [25].

**Immunomodulatory effects of postnatal supplementation with ω-3 LCPUFA**

In contrast to pre- and perinatal interventions, the effects of ω-3 LCPUFA supplementation during infancy on development of T-cell and innate immunity have not been studied previously. Such studies can help shed light on the issue of the optimal timing of allergy preventive interventions. In a recent article of this journal, D’Vaz and colleagues describe the immunomodulatory effects of 650 mg daily fish oil (280 mg docosahexaenoic acid (DHA) and 110 mg eicosapentanoic acid (EPA)) supplementation from birth to six months of age in 150 infants of allergic mothers in a placebo-controlled randomised study [26]. The infants were part of a larger (n=420) study population but were representative of the main trial regarding maternal and paternal age, parity, gestation, birth weight and length, head circumference, household pets gender, delivery mode and season of birth. Direct supplementation, squeezing the content of the fish oil capsules into the mouth of the infant or adding it to milk with the first daily feed, was associated with some difficulty and blinding could not be perfectly achieved, due to the fish oil smell. Postnatal fish oil supplementation did result in higher plasma levels of the ω-3 LCPUFA DHA and EPA and reduced erythrocyte ω-6 arachidonic acid (AA) levels compared with placebo. In agreement with the author’s hypothesis that the supplementation would decrease infant allergy-associated Th2 responsiveness, lower levels of the Th2 cytokine IL-13 was observed in the active than in the placebo group at six months of age after stimulation of peripheral blood mononuclear cells with house dust mite allergen, while the PHA mitogen-induced levels of the Th1 cytokine IFN-γ and the proinflammatory cytokine TNF were increased. Furthermore, high plasma DHA levels were associated with low responses of the Th2 cytokines IL-5
and IL-13 after stimulation with the cow’s milk allergen β-lactoglobulin. In contrast to the author’s hypothesis, the supplementation did not affect IFN-γ-induced HLA-DR expression on monocytes or the numbers of CD4+CD25hiCD127lo regulatory T cells and their Foxp3 expression. Data from flow cytometry analyses were only available from 21 infants, however, limiting the power of these analyses.

**Allergy preventive effects of prenatal, perinatal or postnatal intervention strategies**

Although the effect of postnatal fish oil supplementation on development of eczema should be evaluated for the main cohort of 420 children, the prevalence of eczema at 6 and 12 months of age in the study by D’Vaz et al did not differ between the supplementation groups among these 150 children, despite the decreased Th2 responsiveness to allergens [26]. Other studies on postnatal fish oil supplementation, given directly to the infants [10, 12, 27, 28] or to the lactating mothers [11], have not demonstrated allergy preventive benefits. In contrast, administration of fish oil to mothers during pregnancy [9, 13, 16, 28, 29] or during pregnancy and lactation [14, 15] has shown promising allergy preventive effects. Also, a preventive effect of probiotics on atopic eczema has primarily been demonstrated in studies by us and others where probiotics were given both pre- and postnatally [1-3, 7], whereas studies with postnatal or prenatal supplementation only failed to prevent allergic disease [4-6]. Prenatal probiotic supplementation was not given until 36 weeks of gestation in any of the studies, however [1-3, 6, 7]. If prenatal microbial exposure is vital for the preventive effect, as also suggested by epidemiological [30-32] and experimental [33, 34] studies, probiotic supplementation should maybe be started already from the second trimester of pregnancy, when circulating fetal T cells have developed [7, 8]. In summary, findings from both probiotic and ω-3 supplementation studies support the concept that maternally mediated signals during perinatal critical windows of immune vulnerability may affect immune development in the offspring.

**Dosage of ω-3 LCPUFA supplementation and allergy prevention**
In addition to the timing of intervention, the dosage is also critically important. Which dose of \( \omega-3 \) LCPUFA that is required at various times of dietary interventions to achieve a favourable \( \omega-3/\omega-6 \) LCPUFA ratio in maternal milk and infant serum phospholipids [35] is not understood. With a dosage of 650 mg fish oil daily supplementation, D’Vaz et al clearly influenced the composition of plasma and erythrocyte phospholipids towards a higher \( \omega-3/\omega-6 \) ratio [26]. Although they actually found a positive relationship between a higher EPA/AA ratio in erythrocyte membranes and eczema in the infants, they were not able to demonstrate that the \( \omega-3 \) PUFA supplementation prevented eczema in this subpopulation of the main cohort at 12 months of age [26]. Similarly, in a previous postnatal intervention study [36], infants with heredity for bronchial asthma were supplemented with 500 mg fish oil (180 mg \( \omega-3 \) LCPUFA) from 6 months of age, while at the same time, the study promoted the increased use of \( \omega-3 \) LCPUFA in the household as compared to the children randomized to the placebo group. Increased plasma levels of \( \omega-3 \) LCPUFA were reported in the intervention group at 18 months, 3 and 5 years of age [10, 12, 27, 36]. Neither any relationship between \( \omega-3 \) PUFA plasma levels and allergic disease, nor any preventive effect of the \( \omega-3 \) PUFA supplemements was shown at any time, however [10, 12, 27, 37]. The amount of \( \omega-3 \) LCPUFA supplements used during pregnancy has been substantially higher. Dosages between 1.5 to 4.5 g of fish oil, corresponding to 0.9 g [16], 1.4 g [13], 2.7 g [14] and 3.1 g [9] EPA and DHA, in the intervention group during pregnancy have been shown to substantially change maternal plasma \( \omega-3/\omega-6 \) LCPUFA ratio and show promising allergy preventive effects. Particularly the maternal plasma \( \omega-3 \) LCPUFA levels at birth seem to be an important marker for the development of allergic disease, as the frequency of IgE associated disease at two years of life was reported to decrease with increasing DHA levels in maternal plasma [15]. These dose-related findings clearly need to be further addressed in relation to appropriate timing of interventions in future studies.

**Maternal atopy and allergy preventive strategies**

As the study by D’Vaz and colleagues included allergic mothers only, the influence of maternal atopic heredity on the effect of postnatal fish oil supplementation on
immune and eczema development could not be determined [26]. Interestingly, while the allergy preventive and immunomodulatory effects in our study of maternal fish oil supplementation during pregnancy and lactation seemed to be strongest in infants of non-atopic mothers [15, 23], the preventive effects in our probiotic study were more pronounced in children of atopic mothers where *Lactobacillus reuteri* was administered to mothers from gestational week 36 and to infants during the first year of life [3]. An enhanced effect of maternal and infant probiotic supplementation in children of atopic mothers could suggest that modulatory influences on perinatal immune-mediated signals are particularly important in the presence of maternal allergic inflammation [7]. The more pronounced preventive effect of ω-3 supplementation in non-atopic than atopic mothers on infant allergy development [15] and Th1/Th2 balance [23] could be due to a disturbed polyunsaturated fatty acid metabolism in atopic individuals [38]. As the effects of probiotic and fish oil supplementation seem to vary by maternal atopic status, a combined perinatal supplementation strategy may be more successful. Furthermore, probiotic and ω-3 polyunsaturated fatty acid administration may act synergistically at the cellular level, via immunoregulatory [7] and anti-inflammatory [19] mechanisms, respectively. We are now testing these possible synergistic effects in a new double blind placebo controlled randomized study, PROOM-3, recruiting pregnant women from February 2012 onwards (for details please see ClinicalTrials.gov Identifier NCT01542970).

Pregnant mothers (n=480) from high-risk families will be included in the study at the 20\textsuperscript{th} week of gestation. They will be randomized to 4 study groups. One group will receive placebo for ω-3 and *L reuteri*, the second will receive ω-3 supplementation and placebo regarding *L reuteri*, the third will receive *L reuteri* and placebo regarding ω-3 and the fourth group will receive both ω-3 and *L reuteri* supplementation. ω-3 capsules will be given to mothers during pregnancy and lactation while *L reuteri* oil drops will be given to the mothers during pregnancy and to the children during the first year of life. The children will then be followed to two years of age, the primary outcome being IgE-associated eczema and secondary outcomes will include maternal and infant immunomodulation. Thus, in this study the effect of longer exposure to probiotics during pregnancy and postnatally on allergy prevention can be evaluated, as well as synergistic effects between probiotics and ω-3 supplementation.
Furthermore, the allergy preventive effect of perinatal ω-3 supplementation described in our study by Furuhjelm et al [14, 15] may be confirmed by another independent study. Safety is of course of outmost concern in any studies evaluating perinatal treatment studies. Probiotic and ω-3 supplementation studies during pregnancy, including studies on prevention of preterm delivery, have previously been well tolerated, with low risks for side effects [15, 39-41]. A recent systematic review concluded that ω-3 LCPUFA supplementation during pregnancy resulted in a modest increase in birthweight, a lower risk of early preterm delivery and that occurrence of side effects was generally similar except for belching and bad taste, occurring more frequently in the n-3 LCPUFA-supplemented than the placebo groups [41].

**Epigenetic regulation and prenatal allergy prevention**

Environmental exposures during pregnancy may alter gene expression in the offspring via epigenetic mechanisms, heritable changes in gene expression occurring without alterations in the DNA sequences [42], a kind of cellular memory. Epigenetic modifications determine the degree of DNA compaction and accessibility for gene transcription, thus resulting in changes in gene expression that are subsequently passed to somatic daughter cells during mitosis [42]. The main processes modulating DNA accessibility to establish epigenetic memory occur via posttranslational histone modifications and methylation of DNA CpG dinucleotides [42]. DNA methylation, associated with transcriptional repression, is more rigid than histone modifications, with DNA methyltransferases conferring covalent methyl modifications to evolutionary conserved regulatory gene elements, CpG islands [42]. The methylation pattern is thus preserved with high fidelity through cell divisions, assuring preservation of cellular inheritance [42].

Prenatal environmental exposures may alter gene expression via epigenetic mechanisms, aiming to induce physiological adaptations to the anticipated postnatal environment, but potentially also increasing offspring disease susceptibility [43]. This “Developmental Origins of Health and Disease” hypothesis [43] was originally
proposed by David Barker [44]. Although the importance of fetal programming mostly has been studied in cardiovascular and metabolic disease [43], this hypothesis is also very attractive in the context of environmentally influenced immune-mediated diseases. Developmental plasticity may attempt to match the responses of the offspring to the environment predicted to be experienced via epigenetic mechanisms, but when the responses are mismatched, disease risk may increase [45]. Possibly, interventions that are given both pre- and postnatally may decrease the risk for mismatched responses (Fig). The maternal dietary and microbial environment during pregnancy may program the immune development of the child [17], via epigenetic mechanisms, regulating appropriate maturation of innate immunity [30, 33] and T helper and regulatory responses [46-48]. Th1, Th2 and Th17 differentiation is under epigenetic control and human T regulatory cell commitment requires demethylation of the FOXP3 promoter [49].

Epigenetically regulated childhood immune development by maternal dietary and microbial exposure is likely induced via changes in maternal immune regulation [33, 50], as there is a close immunological interaction between the mother and her offspring during pregnancy [7, 8, 51]. The placenta allows a cross-talk between maternal stimuli, possibly induced via microbial stimulation of maternal Toll-like receptors, and fetal responses [33]. As fetal T cells have developed during the second trimester of gestation, maternal signals may then direct the immune cell lineage commitment of the offspring during a critical developmental period when the epigenetic program is highly susceptible to environmental influences [17]. During pregnancy, the fetal-maternal interface is characterized by high levels of Th2-like cytokines and enrichment of T regulatory cells, diverting the maternal immune response away from damaging Th1-mediated immunity [7]. The association of cord blood IgE levels and neonatal IFN-γ production with maternal but not paternal atopic heredity may depend on an even stronger Th2-deviation in atopic than non-atopic pregnant women [7, 52, 53]. As the cytokine milieu shapes the T helper differentiation, particularly during naïve as compared to established responses [54], the neonatal immune system is Th2-skewed [7, 55]. We have shown an even more marked neonatal Th2-skewing in infants later developing allergic disease [51, 55, 56],
possibly due to prenatal epigenetic effects via maternal immune regulation that may be possible to redress by increasing the dietary $\omega$-3/$\omega$-6 LCPUFA ratio or by enhanced microbial exposure, e.g., via fish oil or probiotic supplementation, during and after pregnancy [8]. The Th2-bias of the new-born should then develop toward a more balanced immune phenotype, including maturation of Th1-like responses [47] and appropriate development of regulatory T cell responses [48]. In farm studies, contact with multiple animal species during pregnancy is positively correlated to Treg cell function and IFN-γ production at birth and with innate immune receptor expression at birth and during childhood [30, 32, 48]. A failure of Th2-silencing during maturation of the immune system may underlie development of Th2-mediated allergic disease [55, 57].

In conclusion, determining the optimal timing of allergy preventive interventions is critical, as early life events occurring during critical windows of immune vulnerability can have long-term impact on immune and allergy development. Increasing knowledge on the immunomodulatory effects of prenatal, perinatal and postnatal interventions, provided by studies such as the recent one by D’Vaz et al [26], will help to direct future strategies to combat the allergy epidemic.

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Figure legend
The maternal dietary and microbial environment during pregnancy may program the immune development of the child via epigenetic mechanisms, regulating appropriate immune maturation. Developmental plasticity may attempt to match the responses of the offspring to the anticipated postnatal environment, but when the responses are mismatched, disease risk may increase. Possibly, immunomodulatory
interventions that are given both pre- and postnatally may decrease the risk for mismatched responses and immune dysregulation.

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Prenatal dietary / microbial immunomodulatory factors

Anticipatory modulation of offspring immunity via epigenetic mechanisms

Mismatched postnatal environment

Dysregulated infant immunity, allergy development

Prenatal dietary / microbial immunomodulatory factors