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High levels of omega-3 fatty acids in milk from omega-3 fatty acid supplemented mothers are related to less immunoglobulin E-associated disease in infancy.

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ABSTRACT

Aim: We previously reported a protective effect of maternal omega-3 fatty acid supplements on the development of immunoglobulin E (IgE) associated disease in infancy. This study assessed omega-3 long chain polyunsaturated fatty acids (LCPUFA) in maternal milk in relation to omega-3 LCPUFA supplementation and the development of allergic disease in their infants.

Methods: This study randomised 95 pregnant women at risk of having an allergic infant, to daily supplements of 2.6g omega-3 LCPUFA or a placebo of 2.7g soy bean oil from gestational week 25 until three months of lactation. Breast milk samples were collected as colostrum, at one and three months. Milk fatty acids were related to allergic outcome in the infants at 24 months.

Results: Omega-3 milk fatty acids were higher in women who received omega-3 supplements than the placebo group ($p < 0.01$). Higher proportions of milk eicosapentaenoic acid and docosahexaenoic acid and a lower arachidonic/eicosapentaenoic acid ratio were associated with an absence of IgE associated disease in the infants. None of the children developed IgE associated atopic eczema below a level of 0.83mol% eicosapentaenoic acid in colostrum.

Conclusion: High omega-3 LCPUFA milk levels in mothers who received omega-3 LCPUFA supplements were related to fewer allergies in their children.

Key Notes

- We randomised 95 pregnant women at risk of having an allergic infant, to daily supplements of omega-3 LCPUFA or a placebo from gestational week 25 until three months of lactation.
- Maternal omega-3 LCPUFA supplementation during pregnancy and lactation substantially increased the levels of milk omega-3 LCPUFA during lactation.
- High omega-3 LCPUFA milk levels in mothers who received the supplements were related to fewer allergies in their children.

Key words: Allergic disease, breastfeeding, immunoglobulin E, omega-3, pregnancy

INTRODUCTION

Environmental exposure during prenatal and postnatal periods seems to be vital in determining susceptibility to atopic diseases in childhood and possibly also in adulthood (1,2). It has been suggested that an increased dietary intake of omega-6 fatty acids, which are abundant in various vegetable oils, and a decreased intake of omega-3 fatty acids, found

in particularly oily fish, have played a part in the increasing incidence of atopic diseases during the past decades (3). This idea is based on the hypothesis that dietary omega-3 long chain polyunsaturated fatty acids (LCPUFA) could reduce the production of pro-inflammatory eicosanoids, such as prostaglandins and leukotrienes, and thereby reduce the risk of atopic disease (4).

Observational studies exploring the consequences of maternal fish intake during pregnancy on atopic or allergic outcomes in their children show protective effects from higher fish intake (5). In addition, although it is controversial (6), introduction to fish early in life also provides protection against development of allergic disease and sensitisation to food and inhalant allergens (7-9).

We have previously reported that low levels of omega-3 LCPUFA and a high arachidonic acid/ eicosapentaenoic acid ratio in mature milk and in serum phospholipids in the infant, appear to be associated with atopic sensitisation and allergy early in life (10,11). Intervention with omega-3 LCPUFA during pregnancy and, or, lactation in order to influence the maturing immune system of the fetus and infant and restrain the development of allergic disease has been reported by us and others (12-15).

Human breast milk provides essential nutrients, including fatty acids, to maintain growth and development of the infant (16). The mother's diet during pregnancy and lactation has an obvious effect on breast milk fatty acid status (16). The relationship between breastfeeding and development of atopic disease in childhood has been controversial for some years (17,18). This could be explained by many variables, such as the variations in the composition of breast milk components influencing the maturation of the infant immune system (19) or the atopic status of the mother (20).

We hypothesised that the preventive effect on the development of infant atopic disease of maternal omega-3 LCPUFA supplementation in our previous study may have been mediated by increased levels of omega-3 LCPUFA in breast milk, due to omega-3

LCPUFA supplementation during pregnancy as well as during lactation. The aims of this study were to assess the levels of LCPUFA in breast milk and to further investigate the association between early exposure to omega-3 LCPUFA during lactation and the development of allergic disease in the infant. We also analysed the findings in relation to the atopic status of the mother.

MATERIAL AND METHODS

Subjects and intervention

Pregnant women in families with a history of allergic disease living in Linköping or Jönköping in the southern part of Sweden were enrolled into an investigator initiated double-blind placebo controlled study that aimed to prevent infant development of allergic disease by maternal supplementation with omega-3 LCPUFA (13). The women were randomly allocated to receive an oral daily supplement of nine capsules a day: 70 received omega-3 fatty acids as 35% eicosapentaenoic acid (1.6 g/day) and 25% docosahexaenoic acid (1.1 g/day) and 75 received a placebo of soya bean oil containing 58% linoleic acid (2.5 g/day) and 6% alpha-linolenic acid (0.28 g/day). The capsules were randomised by the producer (Pharma Nord, Vejle, Denmark). The mothers, as well as the staff handling the clinical and laboratory follow-up, were blinded to the group allocation and the mothers were only identified by their study number. In both preparations, the antioxidant alpha-tocopherol was used in similar amounts, 28 mg/day in the omega-3 group and 36 mg/day in the placebo group, to assure the stability of the oil. Both groups were supplemented from gestational week 25 to three months of lactation for an average of 30.5 weeks (range 15-66 weeks). The methodology used and the clinical outcomes of the study have previously been described and discussed (13).

Colostrum samples were available from 95 of the 145 mother and child pairs who were originally included. Two mothers gave birth to twins, but only the first born in each couple was included. In this study, 42 of these 95 mothers were supplemented with omega-3

LCPUFA and the remaining 53 mothers with soybean oil placebo. The clinical outcome of the supplementation in this subgroup was similar to our previous findings (13). Our findings showed that maternal omega-3 LCPUFA supplementation during pregnancy and lactation decreased the frequency of any immunoglobulin E (IgE) associated diseases in the children up to two years of age when compared with the placebo group. The frequency of IgE associated food allergy in the supplementation group versus the placebo group was 7% versus 23% ($p < 0.05$); the frequency of IgE associated atopic eczema was 12% versus 34% ($p < 0.05$) and the frequency of any IgE associated disease was 12% versus 37% ($p = 0.01$). The total frequency of IgE associated disease among the children of the 95 mothers did not differ from the original, larger group (20.9% versus 21.6%). The frequency of just the clinical symptoms, namely food reactions and, or eczema without sensitisation, were similar in both groups. The descriptive maternal data are provided in Table 1.

The atopic status of the mother was based on a structured interview performed by a trained allergy nurse. The interview covered questions regarding former and present symptoms of atopic eczema, food allergy, allergic rhino-conjunctivitis and bronchial asthma. Maternal serum was collected at inclusion at gestational week 25 and the presence of serum IgE antibodies to a panel of inhalant allergens was analysed with Phadiatop by the UniCap Pharmacia CAP System (Phadia, Uppsala, Sweden) (Table 1). Maternal atopy was defined as previous and, or, current allergic symptoms in combination with a positive Phadiatop screening test (Table 1). We did not include 11 women in our analysis as they could not be defined as atopic according to our definition. Of the remaining 84 women, 56 (67%) were defined as atopic - 25 in the omega-3 group and 31 in the placebo group - and 28 (33%) women were defined as non-atopic, 11 in the omega-3 group and 17 in the placebo group. The study was registered at ClinicalTrials.gov (ID NCT00892684).

Clinical follow up and definitions

The clinical follow up of the children has been previously reported (13). Briefly, the clinical examination was performed by two experienced nurses at three, six and 12 months of age. A paediatrician (CF) was consulted if allergic symptoms were present at any time during the study and performed the clinical assessment of all children at 24 months of age. Both the nurses and paediatricians remained blinded to the intervention throughout the study period.

Skin prick tests were performed at six, 12 and 24 months on the volar aspects of the forearm with skimmed cow's milk, egg white and wheat flour mixed with water. At 24 months of age, timothy and birch were added to the test panel. Soluprick histamine hydrochloride (10mg/ml) served as a positive control and Soluprick albumin diluent as the negative control (ALK-Abello, Hørsholm, Denmark). A wheal diameter of ≥ 3 mm was considered as positive. IgE antibodies towards eggs, milk, cats and wheat were analysed with the ImmunoCAP 100^e Specific IgE (Phadia, Uppsala, Sweden) in serum or plasma samples from the infants at 12 and 24 months of age. Timothy grass and birch were added to the test-panel at 24 months. The detection limit was 0.35 kU/l.

A food reaction was defined as gastrointestinal symptoms, hives, aggravated eczema or wheeze following the ingestion of eggs or milk. Recovery from symptoms after elimination of the particular food from the diet and reoccurrence after reintroduction of the particular food was required for the classification. IgE mediated food reaction was defined as a food reaction and the presence of a food specific positive skin prick test (SPT) and, or, serum IgE antibodies. Eczema was characterised as reoccurring and itching eczematous, lichenified or nummular dermatitis, according to the criteria by Seymore (21) and defined as IgE associated eczema if a positive SPT or detectable IgE antibodies was present. Asthma was defined as doctor-diagnosed wheezing at least three times during the first two years and as IgE associated if there was the presence of IgE antibodies or a positive SPT. Symptoms of

food reaction, eczema or asthma were considered as allergic symptoms and concomitant sensitisation was defined as IgE associated disease.

Milk sample collection

Breast milk samples were collected as colostrum two to four days after partus (n=95) and as mature milk at one month (n=88) and at three months (n=78). Samples at all three points of lactation were taken from 75 mothers. The mothers collected the milk at home in sterile plastic tubes using a manual breast-pump. In order to standardise the sampling, the mothers were instructed to collect the milk during the baby's second meal of the day, two minutes after the baby had started suckling. The samples were immediately frozen at home at -20°C, delivered frozen to the nurse at the three-month control, transported frozen to the laboratory and stored at -70°C until analysis. No sample was thawed on arrival at the laboratory.

Fatty acid analysis

Breast milk lipids were extracted three times according to Folch with chloroform-methanol 2:1 (volume/volume) including 0.01 % butyrate hydroxytoluene (22).

Transmethylation of lipid extract from milk were performed in 3N methanolic-HCl at 80°C over four hours. The fatty acid methyl esters were separated by a 6890N Network GC System gas chromatograph (Agilent Technologies, Stockholm, Sweden) as previously described (23). The fatty acid methyl esters were identified by comparing the retention time of the peaks with those of a known standard, GLC-461 (Nu-Chek Prep. Inc, Minnesota, USA). The amount of each fatty acid was given as molar percentage (mol%) of the total fatty acid content. A control sample, serum from a blood donor and milk sample from a person working within the facility, was analysed in every run. The inter-assay coefficient of variance was typically less than 7%.

Ethical aspects

The study was approved by the Local Ethics Committee at the Faculty of Health Sciences at Linköping University. All families gave written, informed consent.

Statistics

Normal distribution of the data was tested with the one sample Kolmogorov Smirnov test. Fatty acids proportions for the whole group were all normally distributed except for eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid and the arachidonic acid/ eicosapentaenoic acid ratio. When the material was divided based on treatment, all the parameters were normally distributed except for the arachidonic acid/ eicosapentaenoic acid ratio. Normally distributed data sets were calculated with unpaired and paired t-tests. Non-normally distributed data were analysed with the Mann-Whitney U-test and Wilcoxon signed ranks test.

Infants were divided into groups according to the quartile of breast milk omega-3 proportions given to each child and were related to the frequency of infant IgE-associated disease. The extended Mantel-Haenszel chi-square for linear trend was used to assess the difference in prevalence of IgE associated disease between the quartile groups. A difference, in combination with a p-value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS Statistics 19 (IBM Corporation, New York, USA).

RESULTS

In total, 84 (88%) of the 95 children were breastfed exclusively for three months while their mothers were still supplemented with the omega-3 LCPUFA or the placebo (Table 1). The corresponding figure at six months of age was 35%.

Milk PUFA composition during lactation in relation to maternal supplementation.

The proportions of omega-3 LCPUFA; 20:4 omega-3, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid were significantly higher in the colostrum and mature milk from women supplemented with omega-3 LCPUFA at all three sampling occasions than the mothers receiving the placebo (Table 2). The opposite effect, namely lower proportions, was seen with regard to most of the omega-6 LCPUFA in colostrum and mature milk from the mothers who received omega-3 LCPUFA when compared to the placebo supplemented mothers during the same lactation period (Table 2).

The main precursors of both the omega-6 PUFA and omega-3 PUFA series, namely linoleic acid and linoleic acid, increased in proportions between colostrum and mature milk in both groups. The levels of linoleic acid were lower in milk from mothers supplemented with omega-3 fatty acids during the whole lactation period than mothers supplemented with the placebo. This was not seen with regard to the levels of linoleic acid. In contrast, all the omega-6 and omega-3 LCPUFA, except for gamma-linoleic acid (omega-6) and eicosapentaenoic acid (omega-3) were higher in colostrum than mature milk in both groups during the three months of lactation. Gamma-linoleic acid increased between colostrum and mature milk in the placebo supplemented mothers, but this was not the case in the milk of the omega-3 supplemented mothers. Eicosapentaenoic proportions remained high and even increased throughout the lactation period in milk from the omega-3 LCPUFA supplemented mothers and supplementation with omega-3 fatty acids substantially increased the levels of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid in maternal milk during the whole lactation period as compared to placebo (Table 2). The total levels of omega-6 PUFA were lower as well as the total omega-3 PUFA levels were higher in maternal milk during the whole lactation period in omega-3 supplemented mothers. Similarly, the arachidonic acid/ eicosapentaenoic acid ratio was significantly lower in milk from the omega-3 LCPUFA than the placebo supplemented mothers and decreased further between colostrum and mature milk (Table 2).

Milk PUFA levels in relation to IgE associated disease in the children regardless of maternal supplementation

We assessed the relationship between milk PUFA levels in relation to development of disease regardless of supplementation. Infants who develop IgE associated eczema or IgE associated food reaction during their first two years of life were fed with breast milk containing significantly lower proportions of eicosapentaenoic acid and docosahexaenoic acid, both as colostrum and one month mature milk compared to healthy children without IgE sensitisation (Fig. 1). A similar trend was seen in mature milk at three months only for DHA (Fig. 1). Similarly, the arachidonic acid/ eicosapentaenoic acid ratio was significantly higher in colostrum fed to children developing IgE associated atopic disease, but the results were not consistent for mature milk (Fig. 1). We also found that children who were diagnosed with clinical symptoms of eczema or food reaction *without* sensitisation during their first two years of life were fed with breast milk with lower proportions of eicosapentaenoic acid at one month ($p<0.03$) and docosahexaenoic acid in colostrum and one month of lactation ($p<0.02$ for both). A higher arachidonic acid/ eicosapentaenoic acid ratio in milk given by mothers to children who did not develop allergic symptoms did not reach significance.

Milk PUFA levels in relation to clinical symptoms of allergy and, or, sensitisation in the children and to maternal supplementation

To further investigate the relationship between the composition of omega-3 LCPUFA in maternal milk and the development of IgE associated atopic disease in their children, we divided the mother-infant pairs into quartiles regarding breast milk proportions of eicosapentaenoic acid and docosahexaenoic acid and the arachidonic acid/ eicosapentaenoic acid ratio and according to the frequency of IgE associated disease, allergic symptoms without sensitisation and healthy children with or without sensitisation

(Fig. 2). The cumulative incidence of IgE associated disease was lower among children given colostrum with the highest proportions of eicosapentaenoic acid and docosahexaenoic acid and the lowest arachidonic acid/ eicosapentaenoic acid ratio. This was true also for eicosapentaenoic acid (p for trend= 0.02) and docosahexaenoic acid (p for trend= 0.03) proportions in mature milk at one month of lactation but not for the arachidonic acid/ eicosapentaenoic acid ratio at one month nor any fatty acid later.

Eicosapentaenoic acid and docosahexaenoic acid levels in milk of mothers to children with and without IgE associated disease

Based on the results regarding colostrum eicosapentaenoic acid, we formed clusters of infants with or without IgE associated disease regardless of omega-3 supplementation or placebo. We designated the groups A, B and C (Fig.3). The seven infants in group A (0.83-1.29 mol%) were all fed colostrum with the highest proportions of eicosapentaenoic acid and none of these infants were diagnosed with IgE associated disease. The 32 infants in group B (0.27-0.82 mol%) were fed colostrum with intermediate proportions of eicosapentaenoic acid. In this group, five out of 32 (16%) infants were diagnosed with IgE associated disease. Among the 53 infants in group C, fed with the lowest eicosapentaenoic acid colostrum proportions (0.06-0.26 mol%), 17/53 (32%) were diagnosed with IgE associated disease. The number of infants with IgE associated disease were inversely related to colostrum eicosapentaenoic acid proportions (p for trend= 0.04).

All children in group A were born to omega-3 LCPUFA supplemented mothers, as were all but one infant in group B. In group C, all but four infants were randomised to the placebo group. The groups were equal regarding infant sex, birth weight and birth length, number of weeks with supplementation both before and after childbirth, gestational length and breast feeding rates at three months (data not shown).

Thus, in this study, no infant fed on colostrum with eicosapentaenoic acid proportions higher than, or equal to, 0.83 mol% developed IgE associated disease before the age of two years (Fig. 3). Their mothers were all supplemented with omega-3 LCPUFA and 7/42 (17%) of omega-3 LCPUFA supplemented mothers reached this level. The corresponding cut off for docosahexaenoic acid proportions in colostrum was 1.5 mol% and 11/42 (26%) mothers belonging to the omega-3 LCPUFA supplemented group reached proportions above this level. In addition, no child with an arachidonic acid/ eicosapentaenoic acid in colostrum lower than 0.60 developed IgE associated disease before the age of two years. This was true for 21%, (9/42) of the omega-3 LCPUFA supplemented mothers. No mother in the placebo supplemented group reached these high proportions of eicosapentaenoic acid nor docosahexaenoic acid nor low arachidonic acid/ eicosapentaenoic acid. A similar cut-off for human milk collected at one month and three months post-partum was not possible to obtain, possibly due to low number of available samples.

Eicosapentaenoic acid proportions in colostrum were dichotomised in order to determine if maternal atopic status influenced our results. Groups A and B in Figure 3 were assigned to the high eicosapentaenoic acid group and group C to low eicosapentaenoic acid. In the group of non-atopic mothers there was a trend ($p = 0.06$) towards a difference in terms of IgE associated diseases between the groups of infants fed colostrum with high or low proportions of eicosapentaenoic acid. This difference was not present in the group of infants born to mothers with a history of allergy (Table 3). This analysis was not possible to perform for docosahexaenoic acid and arachidonic acid/ eicosapentaenoic acid possibly for the same reasons as above.

DISCUSSION

In this study, we showed that providing omega-3 LCPUFA supplements to mothers during pregnancy and lactation considerably increased the proportions of breast milk omega-3 LCPUFAs when compared to placebo supplemented mothers. Furthermore, high

proportions of omega-3 LCPUFA in colostrum and early mature milk were related to the absence of IgE associated diseases in children at two years of life.

We are reporting similar results to previous studies (20,24) with regard to the composition of both omega-6 and omega-3 PUFA during the first one to two months of lactation. These studies, and our, all showed a tendency to increasing levels of linoleic acid and alpha-linolenic acid as well as decreasing levels of both omega-3 and omega-6 LCPUFA in the milk of mothers receiving placebo or controls.

Breastfeeding as an allergy prevention tool has been extensively debated throughout the years (17). An approach to clarify this controversy has been to study whether the increasing prevalence of allergy in childhood may be related to variations in the composition of various milk components as immunoglobulin A (11,25), cytokines and chemokines (25) and omega-3 and omega-6 milk PUFA (10,20). Such variations could explain the loss of a previously shown prophylactic effect of breastfeeding on allergic symptoms and atopic disease in childhood.

We have previously reported low levels of both omega-3 and omega-6 LCPUFA in the breast milk of atopic than non-atopic mothers (20), but only low levels of the omega-3 LCPUFAs eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid in breast milk during the first three months of lactation were related to the development of allergic disease in the children (5,11). These reports were controversial as other groups (6,26,27) have not been able to find consistently similar results, probably due to clinical follow up based on telephone surveys (6) or questionnaires and, or, home visits (26), the lack of a definition or narrow milk sampling (6,26), small control groups (14 non atopic versus 394 atopic mothers) (27) and the analysis of fatty acids in milk at different points of lactation (6,26,27). However, our study could not find differences between atopic and non-atopic mothers as previously reported (20) in a similar research setting. This may be

explained by either the small sample size among the mothers receiving the placebo (n=53) or by changes in maternal dietary consumption of omega-3 PUFA over time (28).

We hypothesised that dietary omega-3 PUFAs in infancy were related to the development of allergic disease in infancy (3). The relationship between maternal prenatal and, or, postnatal omega-3 LCPUFA supplementation and early development of IgE associated disease is still controversial and has recently been reviewed (29). Although no clear relationship has been seen with single IgE associated diseases, maternal prenatal and, or, postnatal omega-3 LCPUFA supplementation seems to be related to a lower frequency of any IgE related disease, particularly in the first 12-36 months of life. Of eight assessed studies, only the one reported by us (11) supplemented the mothers both during pregnancy and lactation.

The relationship between levels of omega-3 PUFA in breast milk and development of allergic disease in early childhood is controversial (5,6,11,26). That is why we analysed the composition of omega-3 LCPUFA and omega-6 LCPUFA in maternal milk and the relation to the development of allergic disease in the children where early maternal and child omega-3 LCPUFA supplementation through breast feeding decreased the incidence of allergic disease in the children (13). We corroborated our previous findings (11) and showed that this seemed to be a causal relationship as prospective, randomised and double blinded maternal supplementation with omega-3 LCPUFA during pregnancy and lactation not only increased the levels of omega-3 LCPUFA in maternal milk substantially, but also decreased the frequency of IgE-associated disease in infancy (13).

A small study (30) used comparable amounts omega-3 LCPUFA supplementation, namely 1.3g eicosapentaenoic acid and 0.9g docosahexaenoic acid daily from gestational week 30 to 30 days post-partum. Their results showed that the levels of omega-3 LCPUFA were doubled in colostrum, but they also showed that the milk omega-3 LCPUFA levels had

already decreased during the first month of lactation if the supplementation did not continue beyond partum. A later study (31) reported increased concentrations of omega-3 LCPUFA in maternal blood and similar results in milk during supplementation with cod liver oil - 1.3 g eicosapentaenoic acid and 0.9g docosahexaenoic acid daily - during pregnancy and three months of lactation . Supplementation with 1.5 g of omega-3 PUFA to mothers between the first week and the fourth month of lactation significantly increased the levels of milk omega-3 LCPUFA in the supplemented mothers, but the differences were small and late during lactation (24). Two other studies (32,33) supplemented pregnant mothers in order to prevent allergic disease in their children, but neither gave the mothers omega-3 LCPUFA during lactation. In addition, only one (32) reported the milk composition of PUFAs with similar results, as previously reported (30), namely considerably higher levels of omega-3 LCPUFA levels in colostrum from omega-3 LCPUFA supplemented mothers, but clearly decreasing levels later during lactation. Thus, the maternal omega-3 LCPUFA intervention during both pregnancy and lactation in this study resulted in consistently higher proportions of eicosapentaenoic acid and docosahexaenoic acid in breast milk compared to the placebo group during at least three months of lactation. This achieved our goal of not only supplement the mothers, but also the children, with omega-3 LCPUFA during early infancy.

When we clustered the mothers regardless of omega-3 LCPUFA supplementation or not, higher levels of eicosapentaenoic acid and docosahexaenoic acid and lower ratios of in colostrum and mature milk at one month of lactation were related to lower incidence of particularly IgE associated atopic disease, but also to clinical symptoms of allergy, corroborating previous results (11). Furthermore, similarly to our previous report (13) and according to Mahrshahi (15) we divided mother-infant pairs into quartiles based on breast milk proportions of eicosapentaenoic acid, docosahexaenoic acid and the arachidonic acid/ eicosapentaenoic acid ratio. We found close to a dose response relationship between the levels of these omega-3 LCPUFA in colostrum and early mature milk at one month of lactation and the development of IgE associated food allergy and eczema in the children at

two years of age. One could argue that if there is a relationship between maternal milk LCPUFA and atopic outcome in the children regardless of the intervention, the results cannot only be attributed to the supplementation. On the other hand, colostrum eicosapentaenoic acid, docosahexaenoic acid, and particularly arachidonic acid/ eicosapentaenoic acid in the supplementation group, were considerably different, as there was little or no overlapping with the placebo group when it came to attributing the particular distribution to the intervention.

Furthermore, analysis at single milk levels of eicosapentaenoic acid and docosahexaenoic acid in relation to development of IgE associated disease in the single child revealed three groups of children. Firstly there were those with the highest proportions where none of children developed IgE associated disease and all mothers were supplemented with omega-3 LCPUFA. Secondly there were those with the lowest proportions and with the highest frequency of IgE associated disease where all mothers were in the placebo group. Thirdly there was a group that fell between the two, between with moderate proportions of milk omega-3 LCPUFA and a moderate development of IgE associated disease. Thus, it seems that the eicosapentaenoic acid concentration in breast milk that is required to protect infants from developing IgE associated disease is above 0.83 mol% as no child fed colostrum with eicosapentaenoic acid higher than this level developed disease (1.5 mol% for docosahexaenoic acid). Although the development of atopic disease in childhood is multifactorial, our findings corroborate previous reports (11), suggesting a cause-relationship between omega-3 LCPUFA in maternal milk and atopic development in the children.

The mechanisms behind this effect are presently unknown. Maternal fat storage during pregnancy and milk PUFAs during lactation are the main sources for omega-3 and omega-6 PUFA for children. The association between omega-6 LCPUFA with eicosanoids with significantly higher inflammatory potential, the synthesis of omega-3 LCPUFA derived resolvins and protectins - that reduce and actively resolve inflammation in animal models -

and lower blood levels of the Th2 cytokines IL-4 and IL-13 in children supplemented with omega-3 LCPUFA-rich fish oil during pregnancy and lactation have been discussed (34).

CONCLUSION

This study showed that omega-3 LCPUFA supplementation during pregnancy and lactation to mothers with a family history of allergic disease substantially increased the levels of eicosapentaenoic acid and docosahexaenoic acid in maternal milk. Eicosapentaenoic acid and docosahexaenoic acid levels in colostrum and early mature milk were related to the protective effect in the development of IgE associated allergic disease in infancy. Indeed, none of the children developed IgE associated allergic disease when there was more than 0.83 mol% eicosapentaenoic acid or 1.5 mol% docosahexaenoic acid in maternal colostrum.

There are two implications to this result. The first is that preventive studies in the field of atopic disease in childhood should supplement mothers and children with high enough doses of omega-3 LCPUFA to achieve high levels of eicosapentaenoic acid already in colostrum. Supplementation during pregnancy and early lactation seems mandatory and supplementation just during the lactation period is not enough. This would explain some of the controversies in this field. The second is that our results need to be corroborated by further studies to clarify the beneficial effect of omega-3 LCPUFA supplementation, not only in relation to the dose and timing (34), but also in relation to the atopic status of the mother

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Acronyms

LCPUFA: long chain polyunsaturated fatty acids

PUFA: polyunsaturated fatty acids

SPT: skin prick test

IgE : Immunoglobulin E

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Tables

Table 1. Descriptive data of mothers supplemented with 2.7 g omega-3 LCPUFA daily or placebo (soybean oil) from gestational week 25 and during lactation.

	omega-3 (n=42)	placebo (n=53)	
	mean (SD)	mean (SD)	<i>p</i> -value†
Age (years)	31.3 (4.0)	31.8 (4.0)	ns
Gestational length (weeks)	40.2 (1.4)	40.4 (1.2)	ns
No. of weeks with supplementation before partus	15.5 (1.4)	15.7 (1.2)	ns
Total no. of weeks with supplementation	30.8 (5.2)	31.7 (6.6)	ns
	n (%)	n (%)	<i>p</i> -value‡
2 or more children	14 (33)	19 (36)	ns
History of allergic disease	30 (71)	36 (68)	ns
Positive Phadiatop	26 (62)	31 (58)	ns
Exclusive breast feeding at 3 months	37 (88)	47 (89)	ns

† = unpaired *t*-test

‡ = chi-square test

ns = no significant group differences

Table 2. Breast milk fatty acids in colostrum, 1 month and 3 months post partum samples from women supplemented with 2.7 g omega-3 LCPUFA or placebo (soy bean oil) from gestational week 25 to 3 months post partum.

Fatty acid	mol% of total fatty acids								
	colostrum			1 month			3 months		
	omega-3 group (n=42)	placebo group (n=53)	p-value#	omega-3 group (n=39)	placebo group (n=49)	p-value#	omega-3 group (n=34)	placebo group (n=44)	p-value#
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean(SD)	Mean(SD)	
C18:2 ω-6 (LA)	8.39 (1.38)	10.1 (2.13)	<0.01	9.06 (1.59) ^a	10.9 (2.07) ^a	<0.01	10.1 (2.49) ^{a,b}	11.6 (2.38) ^a	<0.01
C18:3 ω-6 (GLA)	0.08 (0.04)	0.08 (0.04)	ns	0.09 (0.03) ^a	0.12 (0.05) ^a	<0.01	0.09 (0.03)	0.12 (0.04) ^a	<0.01
C20:2 ω-6	0.40 (0.11)	0.51 (0.14)	<0.01	0.23 (0.05) ^a	0.26 (0.05) ^a	0.02	0.20 (0.04) ^{a,b}	0.22 (0.04) ^{a,b}	0.02
C20:3 ω-6	0.42 (0.13)	0.51 (0.15)	<0.01	0.27 (0.07) ^a	0.35 (0.09) ^a	<0.01	0.23 (0.04) ^{a,b}	0.29 (0.06) ^{a,b}	<0.01
C20:4 ω-6 (AA)	0.56 (0.12)	0.59 (0.13)	ns	0.39 (0.08) ^a	0.36 (0.07) ^a	ns	0.35 (0.06) ^{a,b}	0.34 (0.06) ^{a,b}	ns
Total ω-6	9.93 (1.32)	11.9 (2.25)	<0.01	10.1 (1.64)	12.0 (2.12)	<0.01	11.0 (2.51) ^a	12.6 (2.42)	0.04
C18:3 ω-3 (LNA)	1.22 (0.27)	1.30 (0.33)	ns	1.45 (0.42) ^a	1.57 (0.54) ^a	ns	1.78 (0.72) ^{a,b}	1.73 (0.46) ^a	ns
C20:4 ω-3	0.24 (0.08)	0.20 (0.09)	<0.01	0.20 (0.07) ^a	0.15 (0.04) ^a	<0.01	0.18 (0.04) ^a	0.13 (0.04) ^{a,b}	<0.01
C20:5 ω-3 (EPA)	0.65 (0.27)	0.13 (0.07)	<0.01	0.77 (0.33) ^a	0.10 (0.04) ^a	<0.01	0.77 (0.42)	0.12 (0.05)	<0.01
C22:5 ω-3 (DPA)	0.84 (0.36)	0.31 (0.10)	<0.01	0.55 (0.20) ^a	0.20 (0.05) ^a	<0.01	0.47 (0.19) ^{a,b}	0.19 (0.06) ^a	<0.01
C22:6 ω-3 (DHA)	1.27 (0.41)	0.53 (0.20)	<0.01	1.11 (0.43) ^a	0.27 (0.10) ^a	<0.01	0.98 (0.50) ^a	0.27 (0.14) ^a	<0.01
Total ω-3	4.23 (1.00)	2.46 (0.54)	<0.01	4.07 (1.06) ^a	2.30 (0.56)	<0.01	4.18 (1.44)	2.44 (0.57)	<0.01
AA/EPA‡	0.83 (0.64-1.17)	5.67 (3.76-6.87)	<0.01	0.50 (0.42-0.71) ^a	3.77 (3.20-4.40) ^a	<0.01	0.42 (0.33-0.78) ^a	3.10 (2.45-3.99) ^a	<0.01
AA/DHA‡	0.40 (0.40-0.50)	1.10 (0.90-1.50)	<0.01	0.40 (0.30-0.50) ^a	1.30 (1.10-1.80) ^a	<0.01	0.30 (0.30-0.62)	1.40 (1.10-1.80) ^a	<0.01

LCPUFA, long chain polyunsaturated fatty acids; LA, linoleic acid; GLA, gamma linolenic acid; AA, arachidonic acid; LNA, alpha linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid

#, t-test

‡, not normally distributed, median (first and third quartiles), tested with non-parametric tests, Mann-Whitney U-test or Wilcoxon signed ranks test

a, significantly different from colostrum sample, t-test

b, significantly different from 1 month sample, t-test

ns, no significant difference between groups

Table 3. Main clinical outcomes, divided into groups according to maternal allergic history and colostrum EPA proportions, in children born to mothers supplemented with 2.7 g/d omega-3 LCPUFA or 2.8g/d placebo (soybean oil) from gestational week 25 to 3 months lactation.

Cumulative incidence	No maternal history of allergy			Maternal history of allergy		
	High EPA	Low EPA		High EPO	Low EPA	
Infants 0-24 months	n (%)	n (%)	p‡	n (%)	n (%)	p‡
IgE mediated food allergy †	0/11 (0)	6/18 (33)	0.06	3/28 (11)	6/37 (16)	0.72
IgE associated eczema ¶	0/11 (0)	6/18 (33)	0.06	4/28 (14)	8/36 (22)	0.53
IgE associated disease §	1/11 (9)	7/17 (41)	0.1	4/28 (14)	10/36 (36)	0.23

High EPA = 0.27-1.29 mol%

Low EPA = 0.06-0.26 mol%

† = Gastrointestinal symptoms, hives, aggravated eczema or wheeze following the ingestion of egg or milk. Recovery from symptoms after the elimination of the particular food from the diet and reoccurrence after re-ingestion of the particular food in combination with the presence of food specific positive skin prick test and/or serum IgE antibodies.

¶ = Reoccurring and itching eczematous, lichenified or nummular dermatitis according to the criteria by Seymore (21) in combination with the presence of positive skin prick test and/or serum IgE antibodies.

§ = Symptoms of food reaction, eczema, asthma or rhino-conjunctivitis with positive skin prick test or serum IgE antibodies.

‡ = Chi-square test (Fisher's exact test if expected values were less than 5).

Figure legends

Figure 1. Infants who did not develop IgE associated eczema or IgE associated food reaction during their first two years had received breast milk with higher proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and breast milk with a lower arachidonic acid (AA)/EPA ratio than infants that developed IgE associated eczema or food reaction. The 10th, 25th, 50th, 75th and 90th percentiles are indicated.

Figure 2. Mother-infant pairs divided into four groups according to quartiles of colostral a) eicosapentaenoic acid (EPA), b) docosahexaenoic acid (DHA) proportions and c) arachidonic acid (AA)/EPA ratios in colostrum from mothers supplemented with 1.6 g eicosapentaenoic acid (EPA) and 1.1 g docosahexaenoic acid (DHA) daily or placebo (soybean oil) from gestational week 25 until three months of lactation. The presence of allergic symptoms and, or sensitisation is presented cumulative to 2 years of age.

Black bars: Infants with IgE associated disease (allergic symptoms combined with positive skin prick test (SPT) and/or circulating specific IgE).

Light grey bars: Infants with allergic symptoms but no positive SPTs or detectable circulating specific IgE.

Dark grey bars: Infants without clinical symptoms but with positive SPT or detectable circulating specific IgE.

White bars: Infants without any allergic symptoms or sensitisation. Hence, the outcome of allergic disease and, or allergic sensitisation in all children in each quartile is represented. Extended Mantel-Haenszel chi square for linear trend was used to assess the difference in prevalence of IgE-associated disease between the quartile groups.

Figure 3. Distribution of eicosapentaenoic acid (EPA) proportions in colostrum given to infants with and without IgE associated disease cumulative to two years of age. The mothers were supplemented with 1.6 g eicosapentaenoic acid (EPA) and 1.1 g docosahexaenoic acid (DHA) daily (open dots) or placebo (dark dots) (soybean oil) from gestational week 25 and during three months of lactation. No infant fed colostrum with eicosapentaenoic acid (EPA) proportions higher than or equal to 0.83

mol% developed IgE associated disease before the age of two years. Infants in group A were all fed the highest proportion of eicosapentaenoic acid (EPA) in colostrum by omega-3 LCPUFA supplemented mothers and no infant expressed signs of IgE associated disease. Group B comprise of infants fed colostrum with intermediate proportions of eicosapentaenoic acid (EPA), all but one belong to the omega-3 LCPUFA supplemented group. Five out of 32 (16%) infants were diagnosed with IgE associated disease. In group C, with the lowest proportions of eicosapentaenoic acid (EPA), 21/53 (32%) infants were diagnosed with IgE associated disease and in this group all but four belonged to the placebo group.

Figure 1.

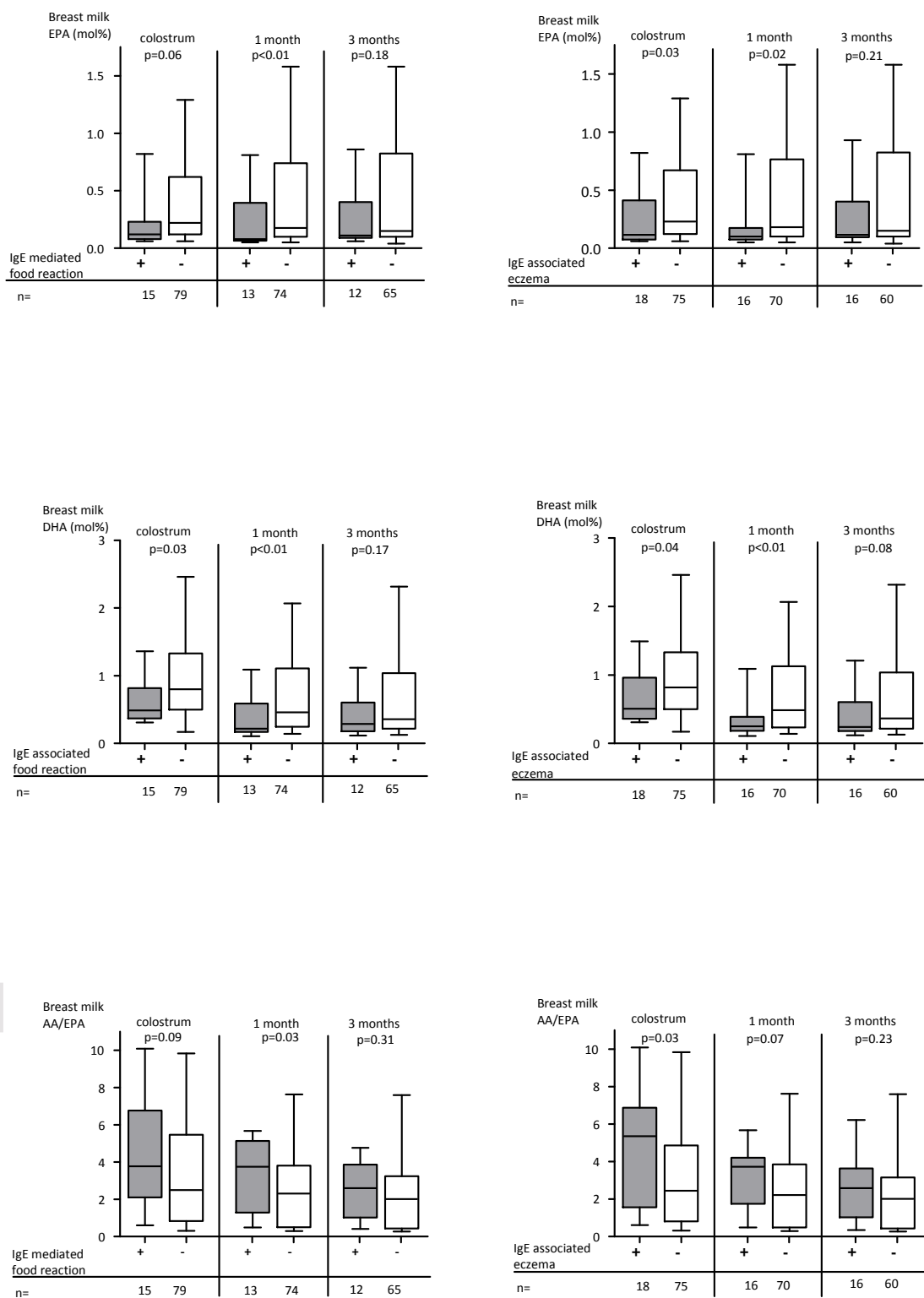
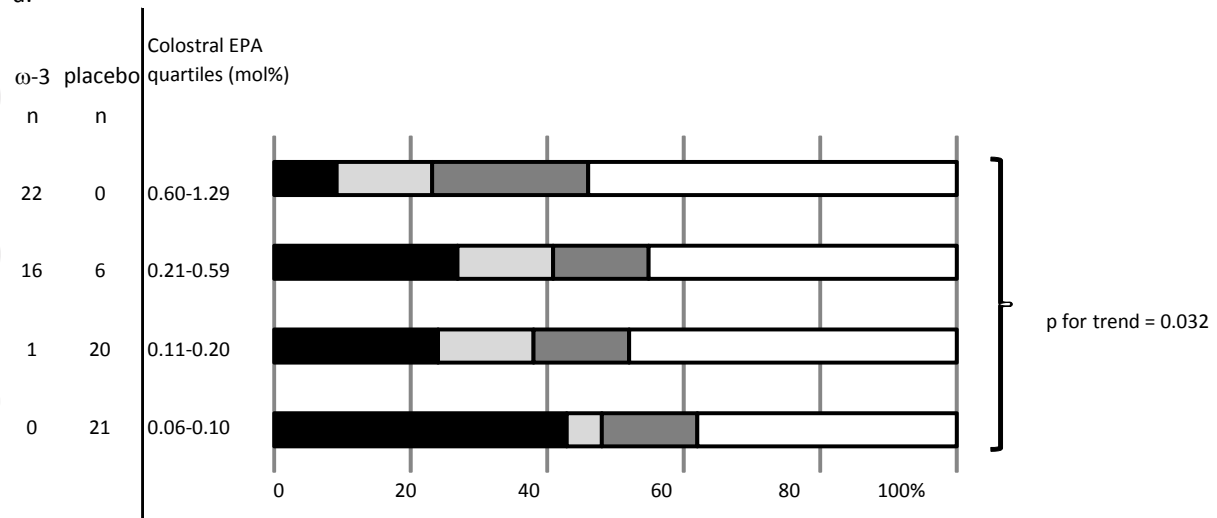
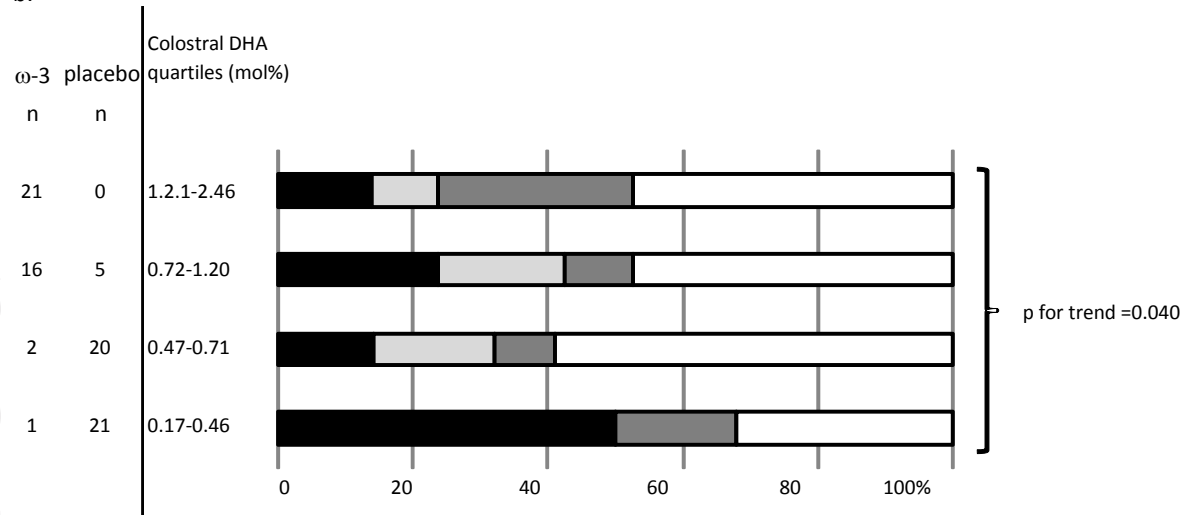


Figure 2 .

a.



b.



c.

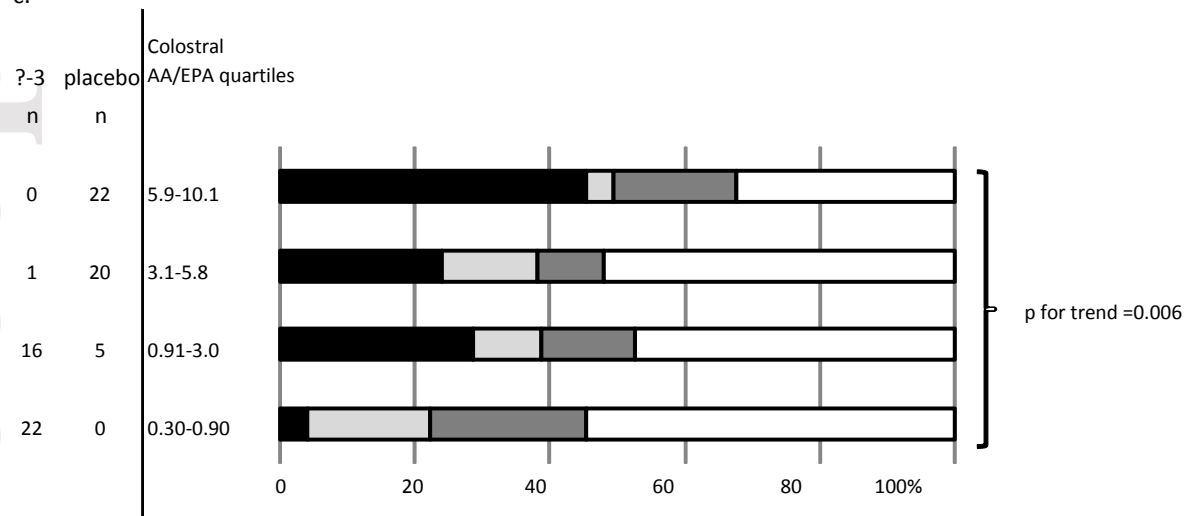


Figure 3.

