Title: Randomised controlled trial of fish oil supplementation in pregnancy on childhood allergies.

Short title: Fish oil in pregnancy and childhood allergies.

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Abstract

Background: Diets high in n-3 long chain polyunsaturated fatty acids (LCPUFA) may modulate the development of IgE-mediated allergic disease and have been proposed as a possible allergy prevention strategy. The aim of this study was to determine whether n-3 LCPUFA supplementation of pregnant women reduces IgE-mediated allergic disease in their children.

Methods: Follow-up of children (n=706) at hereditary risk of allergic disease in the Docosahexaenoic Acid to Optimise Mother Infant Outcome randomised controlled trial. The intervention group (n=368) was randomly allocated to receive fish oil capsules (providing 900 mg of n-3 LCPUFA daily) from 21 weeks' gestation until birth; the control group (n=338) received matched vegetable oil capsules without n-3 LCPUFA. The diagnosis of allergic disease was made during medical assessments at 1 and 3 years of age.

Results: No differences were seen in the overall percentage of children with IgE-mediated allergic disease in the first 3 years of life between the n-3 LCPUFA and control groups (64/368 (17.3%) *v* 76/338 (22.6%); adjusted relative risk 0.78; 95% CI 0.58 to 1.06; *P*=0.11). Eczema was the most common allergic disease; 13.8% of children in the n-3 LCPUFA group had eczema with sensitisation compared with 19.0% in the control group (adjusted relative risk 0.75; 95% CI 0.53 to 1.05; *P*=0.10).

Conclusions: Overall n-3 LCPUFA supplementation during pregnancy did not significantly reduce IgE-associated allergic disease in the first three years of life. Further studies should examine whether the non-significant reductions in IgE-associated allergies are of clinical and public health significance.

Key words

Allergy prevention; eczema; fatty acids; pregnancy; randomised controlled trial.

Abbreviations

AA - arachidonic acid

DHA - docosahexaenoic acid

DOMInO - Docosahexaenoic acid to Optimise Mother Infant Outcome

EPA - eicosapentaenoic acid

IgE - immunoglobulin E

PUFA - polyunsaturated fatty acids

RCT - randomised controlled trial

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Introduction

Changing dietary patterns have favoured increased intake of n-6 polyunsaturated fatty acids (PUFA) from linoleic acid (18:2, n-6) rich vegetable oils, especially since margarine and vegetable oils have became a common dietary staples over the past 40 years. This, together with an increase in consumption of meat-based products, has led to an increase of arachidonic acid (AA, 20:4, n-6) in tissues. AA gives rise to eicosanoids such as prostaglandin E_2 that can enhance the synthesis of T helper type 2 cytokines and immunoglobulin E (IgE) antibodies potentially leading to sensitisation to allergens. However when diets are high in n-3 long-chain (LC) PUFA (from fatty fish and fish oils) they are readily incorporated into cellular phospholipids and thereby displace AA and alter membrane composition and fluidity. This leads to a range of immunological effects, including reduction of prostaglandin E_2 synthesis (1, 2) providing a plausible mechanism by which diets high in n-3 LCPUFA may modulate the development of IgE-mediated allergic disease.

In support of this biological mechanism, epidemiological studies have reported that increased maternal fish intake during pregnancy is associated with reduced atopic or allergic outcomes in children (3-7). Furthermore, evidence from randomised controlled trials (RCTs) (8-11) involving fish oil supplementation of pregnant women have found beneficial effects on reduced allergen sensitisation and allergic disease outcomes in the offspring. However previous RCTs have only reported allergic disease outcomes at 1 year (8, 9), 2 years (12) and 16 years of age (10) and none have reported outcomes between 2 and 16 years of age.

Our study was specifically designed to assess the effect of n-3 LCPUFA supplementation, predominantly as DHA, in pregnancy on the cumulative incidence of IgE-mediated allergic

disease in the first 3 years of life. Outcome data focussing on eczema and food allergy over the first year of life have been previously published (11).

Methods

Subjects and study design

The present study is an allergy follow-up of a subset of children whose mothers were participants in the Docosahexaenoic Acid (DHA) to Optimise Mother Infant Outcome (DOMInO) Trial (13). This subset of children all had a mother, father or sibling with a history of medically diagnosed allergic disease (asthma, allergic rhinitis, eczema) and they were enrolled at either of the two study centres in Adelaide, Australia. Full details of entry into this allergy follow-up study have been previously published (11). Written informed consent was sought before birth and included consent for their offspring to participate in both the 1 and 3 year of age allergy follow-up assessments. Approval for this study was granted by the Human Research Ethics Committees of each centre, Women's and Children's Hospital, Adelaide and Flinders Medical Centre, Adelaide. This allergy follow-up study was registered at www.anzctr.org.au as ACTRN12610000735055. The Docosahexaenoic Acid to Optimise Mother Infant Outcome (DOMInO) trial was registered at www.anzctr.org.au as ACTRN12605000569606.

The dietary treatments for the DOMInO trial have been previously described (13). Briefly, women allocated to the n-3 LCPUFA group were asked to consume three 500 mg capsules of fish oil concentrate, providing 800 mg of DHA and 100 mg of eicosapentaenoic acid (EPA); women in the control group were asked to take three 500 mg vegetable oil capsules without n-3 LCPUFA daily. This was a double-blinded study; all capsules were similar in size, shape and colour. Women took capsules from 21 weeks' gestation until delivery.

Early childhood allergic disease outcome assessments and definitions

The primary outcome was diagnosis of IgE-associated allergic disease (eczema, asthma, allergic rhinitis or food allergy) at 1 or 3 years of age. Participating children attended a medical review appointment at 1 and 3 years of age, conducted by one of six medical practitioners (who made the allergic disease diagnosis for a period covering the previous 12 months by taking a structured history and doing a standardised clinical examination) and one of six experienced research nurses (who performed skin prick testing). All were blinded to treatment group allocation and had quality assurance reviews every six months with one of the investigators (DJP). Data were also collected on possible confounding variables, including details of number of other children in the home, use of house dust mite covers for mattresses and pillows, presence of a cat as a pet and household smoking exposure.

Sensitisation was defined as a positive skin prick test (wheal ≥3mm above negative control) to at least one of the allergens assessed. At 1 year of age, the food allergens tested were whole hens' egg, cows' milk, wheat, tuna and peanut, and the aeroallergens tested were ryegrass pollen, olive tree pollen, *Alternaria tenuis*, cat hair and house dust mite (*Dermatophagoides pteronyssinus)*. At 3 years of age, the same allergens were tested with the addition of two foods (cashew nut and sesame seed) and one aeroallergen (house dust mite, *Dermatophagoides farinae*). The cow's milk allergen extract became unavailable from the supplier for an extended period during the 3 year assessments and consequently was excluded from the definition of sensitisation at 3 years.

Eczema was defined as the presence of eczema (criteria according to (14)) on medical review or a history of an itchy rash distributed to the facial, flexural, or extensor surface of the skin that had followed a fluctuating or chronic course. We defined IgE-associated eczema or atopic eczema as eczema with sensitisation to at least one of the allergens assessed. IgE-

associated food allergy was defined as a history within the of immediate (within 60 minutes) skin rash (hives, rash, or swelling) with or without respiratory symptoms (cough, wheeze, stridor), gastrointestinal symptoms (abdominal pain, vomiting, loose stools), or cardiovascular symptoms (collapse) following ingestion of a food and sensitisation to the implicated food. Asthma was defined a history of 3 or more episodes of wheeze with the episodes less than 6 weeks apart and/or daily use of asthma medication. Allergic rhinitis was defined as a history of sneezing, or a runny, or blocked nose accompanied by itchy-watery eyes when there have not been symptoms to suggest an upper respiratory tract infection. IgE-associated asthma/allergic rhinitis was defined as asthma/allergic rhinitis along with sensitisation to at least one of the aeroallergens tested.

Statistical methods

With >328 children per treatment group we would be able to detect an absolute reduction of 10% (relative reduction of 33%) in the cumulative incidence of IgE-mediated allergic disease from 30% to 20% with >80% power ($\alpha = 0.05$) over the first 3 years of life. Such a reduction was realistic based on the pilot data from Dunstan *et al* (8).

All analyses were performed according to the intention to treat principle. Multiple imputation was used to deal with missing data, with 50 complete datasets imputed for analysis. Overall the missing at random assumption of the multiple imputation approach appeared reasonable for these data. Binary outcomes were analysed using log binomial regression models, with treatment effects expressed as relative risks. Rare binary outcomes were compared between groups using Fisher's exact tests on the original (unimputed) data. Negative binomial regression models were used to analyse count outcomes, with the effect of treatment expressed as a ratio of means. Both unadjusted and adjusted analyses were

performed, with adjustment for the stratification variables of centre and parity as well as the pre-specified baseline variables of infant sex and maternal history of allergic disease. The adjusted analyses were considered to be the primary analyses. Potential confounding variables (environmental characteristics measured after randomization) that may influence allergic disease outcomes were compared between groups using Mann Whitney and chi square tests. Statistical significance was assessed at the two sided $P<0.05$ level. All analyses were performed using SAS version 9.3.

Results

The allergy follow-up trial profile is shown in Figure 1. Enrolment began on 20th March 2006 and ended on 8th May 2008, with a total of 706 infants recruited into the study. Data collection for the medical assessments at 3 years of age was completed on 1st September 2011. 638/706 (90.4%) children attended a medical review at 3 years of age with 587/706 (83.1%) of children having skin prick testing to determine their sensitisation status.

Demographic and family characteristics

The participants consisted of 337/706 (47.7%) males and 281/706 (39.8%) were the first born (parity zero). Mean maternal age at trial entry was 29.6 years (SD 5.7 years) and 92/706 (13.0%) of the participating mothers smoked during pregnancy. All participants had at least one first degree relative with a history of medically diagnosed allergic disease; 656/706 (92.9%) had a least one parent and 206/706 (29.2%) had both parents with a history of allergic disease. Other baseline demographic characteristics of the trial participants and their families have been previously reported (11). Early childhood (0-3 years) home environmental characteristics are shown in Table 1; there were no statistically significant differences between the groups.

Early Childhood Allergic Disease Outcomes

In the n-3 LCPUFA intervention group 17.3% of children were diagnosed with IgE-mediated allergic disease (asthma, allergic rhinitis, eczema and/or food allergy) in the first 3 years of life compared with 22.6% in the control group (adjusted relative risk 0.78; 95% CI 0.58 to 1.06; P=0.11; Table 2). As expected, the most common IgE-mediated allergic disease in the

first 3 years of life was eczema with sensitisation, affecting a total of 115/706 (16.3%) of the participating children. There was a lower, but not statistically significant, incidence of eczema with sensitisation in the n-3 LCPUFA group (13.8% vs 19.0% control group, adjusted relative risk 0.75; 95% CI 0.53 to 1.05; *P*=0.10; Table 2). Overall 4.6% of the children were diagnosed with at least one IgE-mediated food allergy through to 3 years of age and egg allergy was the most common, affecting 2.8% of the children. There were no differences between the n-3 LCPUFA and control groups in the percentage of children diagnosed with food allergy or respiratory allergic diseases (asthma, allergic rhinitis) with sensitisation through to 3 years of age (Table 2). The percentage of children diagnosed with allergic disease without sensitisation (non IgE-mediated) did not differ between groups at either time point (Tables 2 and 3).

Table 3 reports the allergic disease outcomes at 3 years of age and demonstrates that there were no differences between the n-3 LCPUFA and control groups. The percentage of children diagnosed with eczema with sensitisation at 3 years of age was 13.0% and higher than that reported at 1 year of age (9.2% (11)). Of the 92 children diagnosed with IgE-associated eczema at 3 years of age, 54% were new cases (25 in each group) who did not have IgE-associated eczema at 1 year of age.

There was no difference between the groups in sensitisation to at least one allergen at 1 or 3 years of age, with 29.4% of children in the n-3 LCPUFA group sensitised compared with 35.2% in the control group (adjusted relative risk 0.85; 95% CI 0.68 to 1.06; *P*=0.14; Table 2). At 3 years of age, 24.6% of children in the n-3 LCPUFA group compared with 26.1% in the control group were sensitised to at least one allergen (adjusted relative risk 0.96; 95% CI

0.74 to 1.25; *P*=0.76; Table 4). Alternaria Tenius (grass mould) (total of 7.8%), ryegrass pollen (total of 7.1%) and D. Pteronyssinus (total of 6.8%) were the most common allergens that children were sensitized to at 3 years (Table 4). Despite no differences in cat ownership between the groups in the first 3 years of life (Table 1), 6.8% of children in the n-3 LCPUFA group compared to 3.4% in the control group were sensitised to cat at 3 years of age, however this difference did not reach statistical significance (adjusted relative risk = 1.95; 95% CI 0.98 to 3.89; *P*=0.06; Table 4). Figure 2 illustrates the changing profile of sensitisation status from predominately food allergen sensitisation at 1 year of age to increased aeroallergen sensitisation at 3 years of age. Egg sensitisation was found in a total of 86/706 (12.2%) of the children at 1 year of age but only 27/706 (3.8%) at 3 years of age.

Discussion

This study is the largest randomised controlled trial of fish oil supplementation during pregnancy and was designed to resolve uncertainties surrounding the use of n-3 LCPUFA supplementation in pregnancy as an allergic disease preventative strategy for children with hereditary risk. Overall fish oil supplementation in pregnancy did not significantly reduce the cumulative incidence of IgE-associated allergic disease over the first 3 years of life. Our study was powered to detect a 33% relative reduction in allergic disease and it is therefore not surprising that the differences noted did not reach statistical significance. The non-significant risk reductions of up to 22% may still be of public health significance as the burden and cost of allergic disease on affected families is high and fish oil intervention is safe and relatively cheap.

The lower incidence of IgE-associated eczema observed at 1 year of age in the n-3 LCPUFA group did not persist at 3 years of age. As more than half of the children diagnosed with eczema with sensitisation at 3 years of age did not have this diagnosis at 1 year of age, it is interesting to question whether these new cases could have been reduced if the n-3 LCPUFA supplementation had continued beyond pregnancy during early childhood. A recent postnatal (0-6 months of age) n-3 LCPUFA supplementation trial (n= 420) (15) found no overall effect on allergic disease outcomes at 1 year of age, but there was a significant reduction in eczema diagnosis in those infants who had higher n-3 LCPUFA levels at 6 months of age. This may be an important influencing factor as Furuhjelm et al (12) (n-3 LCPUFA supplementation during pregnancy and first 3.5 months of lactation) also found higher maternal and infant proportions of DHA and EPA in plasma phospholipids were associated with lower prevalence of IgE-associated allergic disease in a dose-dependent manner. Interestingly compared with our study, other studies have used higher doses of n-3 LCPUFA that have ranged from

2700mg/day (10,12) to 3700mg/day (8) and it may be that higher n-3 LCPUFA doses are needed to result in reduced allergic disease outcomes. Dose, timing and duration of n-3 LCPUFA supplementation are important considerations and worthy of further investigation. A limitation of our study was that we have not taken blood samples from the children and hence cannot report on their n-3 LCPUFA plasma phospholipids levels at 1 or 3 years of age.

The pattern of allergic disease is known to differ with age, with the greatest incidence of food allergy and atopic dermatitis/eczema being in the first few years of life, while asthma and allergic rhinitis continue to rise until adulthood (16). Similarly the changing sensitisation pattern was as expected; the incidence of food allergen sensitisation decreased and the aeroallergen sensitisation increased between 1 to 3 years of age (17, 18). Specifically, the frequency of egg sensitisation significantly reduced and the significant reduction in egg sensitisation at 1 year of age with n-3 LCPUFA treatment (9.3% vs 15.4%; adjusted relative risk 0.62; 95% CI 0.41 to 0.93; *P*=0.02) (11) disappeared by 3 years of age. One could question whether the timing of various allergen exposures during the first few years of life and the corresponding timing of the n-3 LCPUFA supplementation may influence the pattern of sensitisation.

Conclusion

Overall n-3 LCPUFA supplementation during pregnancy did not result in a significant reduction in IgE-associated allergic disease in the first three years of life to the magnitude originally predicted. Collectively, results from this and other n-3 LCPUFA supplementation RCTs suggest that the dose, timing and duration of n-3 LCPUFA supplementation may

influence sensitisation and allergic disease outcomes. Clearly further follow up studies are required to definitively determine whether there is benefit in n-3 LCPUFA supplementation as an allergy prevention strategy.

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Authors Contributions:

Conception and design (MM, MG, SP, RG), acquisition of data (DP, MM, TS, MG, SP, RH), analysis and interpretation of data (DP, MM, TS, MG, SP, RH, RG), drafting of the manuscript (DP, MM, TS), critical revision of the manuscript (all), statistical analysis (DP, MM, TS), obtained funding (MM, MG, SP, RG, DP) and study supervision (DP, MM, MG, RH).

References

1. Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. *Br J Nutr* 2002;**87** Suppl 1:S31-48.

2. Gottrand F. Long-chain polyunsaturated fatty acids influence the immune system of infants. *J Nutr* 2008;**138**(9):1807S-1812S.

3. Calvani M, Alessandri C, Sopo SM, Panetta V, Pingitore G, Tripodi S, et al. Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. *Pediatr Allergy Immunol* 2006;**17**(2):94-102.

4. Romieu I, Torrent M, Garcia-Esteban R, Ferrer C, Ribas-Fito N, Anto JM, et al. Maternal fish intake during pregnancy and atopy and asthma in infancy. *Clin Exp Allergy* 2007;**37**(4):518-525.

5. Salam MT, Li YF, Langholz B, Gilliland FD. Maternal fish consumption during pregnancy and risk of early childhood asthma. *J Asthma* 2005;**42**(6):513-518.

6. Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, et al. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. *Am J Clin Nutr* 2007;**85**(2):530-537.

7. Willers SM, Devereux G, Craig LC, McNeill G, Wijga AH, Abou El-Magd W, et al. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 2007;**62**(9):773-779.

8. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003;**112**(6):1178-1184.

9. Furuhjelm C, Warstedt K, Larsson J, Fredriksson M, Bottcher MF, Falth-Magnusson K, et al. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatr* 2009;**98**(9):1461-1467.

10. Olsen SF, Osterdal ML, Salvig JD, Mortensen LM, Rytter D, Secher NJ, et al. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am J Clin Nutr* 2008;**88**(1):167-175.

11. Palmer DJ, Sullivan T, Gold MS, Prescott SL, Heddle R, Gibson RA, et al. Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ* 2012;**344**:e184.

12. Furuhjelm C, Warstedt K, Fageras M, Falth-Magnusson K, Larsson J, Fredriksson M, et al. Allergic disease in infants up to 2 years of age in relation to plasma omega-3 fatty acids and maternal fish oil supplementation in pregnancy and lactation. *Pediatr Allergy Immunol* 2011;**22**(5):505-514.

13. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. *JAMA* 2010;**304**(15):1675-1683.

14. Hanifin JM, Rijka G. Diagnostic features of atopic dermatitis. *Acta Dermatovener* 1980;**92**:44-47.

15. D'Vaz N, Meldrum SJ, Dunstan JA, Martino D, McCarthy S, Metcalfe J, et al. Postnatal fish oil supplementation in high-risk infants to prevent allergy: randomized controlled trial. *Pediatrics* 2012;**130**(4):674-682.

16. Spergel JM. From atopic dermatitis to asthma: the atopic march. *Ann Allergy Asthma Immunol* 2010;**105**(2):99-106; quiz 107-9, 117.

17. Dean T, Venter C, Pereira B, Arshad SH, Grundy J, Clayton CB, et al. Patterns of sensitization to food and aeroallergens in the first 3 years of life. *J Allergy Clin Immunol* 2007;**120**(5):1166-1171.

18. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999;**103**(6):1173-1179.

Figure 1: Trial participant outcomes flow diagram

Figure 2: Allergen Sensitisation Profile at 1 and 3 years of age