

Prenatal Fish Oil Supplementation and Allergy: 6-Year Follow-up of a Randomized Controlled Trial

Karen P. Best, RN, RM, PhD,^{a,b,c} Thomas Sullivan, BMA&CompSc, BSocSc,^d Debra Palmer, BSc, BND, PhD,^{b,e} Michael Gold, MBChB, FRACP, MD,^c Declan (John) Kennedy, MD, FRCP, FRACP,^{c,f} James Martin, MBChB,^f Maria Makrides, BSc, BND, PhD^{a,b,c}

abstract

BACKGROUND AND OBJECTIVE: Evidence from randomized controlled trials in early infancy suggest that prenatal supplementation with Ω -3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA) reduces the incidence of allergic disease characterized by an immunoglobulin E (IgE) response. We aimed to determine whether protective effects were evident in the 6-year-old offspring of women supplemented with n-3 rich fish oil during pregnancy.

METHODS: Six-year follow-up of children ($n = 706$) with a family history of allergic disease from the Docosahexaenoic Acid to Optimize Mother Infant Outcome (DOMInO) trial. Women were randomly allocated to receive n-3 LCPUFA-rich fish oil capsules (800 mg/d docosahexaenoic acid DHA and 100mg/d eicosapentaenoic acid) or vegetable oil capsules (without n-3 LCPUFA). Allergic disease symptoms including eczema, wheeze, rhinitis, and rhino-conjunctivitis, were assessed using the International Study of Asthma and Allergies in Childhood questionnaire and sensitization to allergens was measured by skin prick test.

RESULTS: There was no difference in the percentage of children with any IgE-associated allergic disease between the n-3 LCPUFA and control groups (116/367 [31.5%] vs 106/336 [31.5%]; adjusted relative risk, 1.04; 95% confidence interval, 0.82–1.33; $P = .73$). There was a reduction in the percentage of children sensitized to house dust mite *Dermatophagoides farinae* (49/367 [13.4%] vs 68/336 [20.3%]; adjusted relative risk, 0.67, 95% confidence interval, 0.44–1.00; $P = .0495$).

CONCLUSIONS: Prenatal n-3 LCPUFA supplementation did not reduce IgE-associated allergic disease at 6 years of age. Secondary outcomes were suggestive of a protective effect of the intervention on the incidence of *D. farinae* sensitization.



^aHealthy Mothers, Babies and Children, South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia; ^bWomen's & Children's Health Research Institute, ^cDiscipline of Paediatrics, School of Medicine, and ^dSchool of Population Health, University of Adelaide, Adelaide, South Australia, Australia; ^eSchool of Paediatrics and Child Health, University of Western Australia, Subiaco, Western Australia, Australia; and ^fDepartment of Respiratory and Sleep Medicine, Women's & Children's Hospital, North Adelaide, South Australia, Australia

Dr Best designed the study and data collection instruments, coordinated and supervised data collection, and drafted the initial manuscript; Mr Sullivan designed the statistical analysis plan, performed the analyses, and critically reviewed the manuscript; Dr Palmer and A/Prof Gold conceptualized and designed the study, designed the data collection instruments, and critically reviewed the manuscript; Prof Kennedy and Dr Martin designed the study and critically reviewed the manuscript; Prof Makrides conceptualized and designed the study, designed the data collection instruments and statistical analysis plan, and reviewed the manuscript; and all authors approved the final manuscript as submitted.

This trial has been registered with the Australian New Zealand Clinical Trial Registry (<http://www.anzctr.org.au/>) (identifier 12615000498594).

WHAT'S KNOWN ON THIS SUBJECT: Evidence suggests prenatal n-3 LCPUFA causes modulation of the fetal immune system and decreased incidence of allergic disease.

WHAT THIS STUDY ADDS: This study will determine whether prenatal n-3 LCPUFA supplementation reduces symptoms of allergy in the 6 year old child with familial risk.

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The remarkable increase in allergic disease prevalence over the last 30 years is a significant global health problem that contributes to deficits in quality of life and, in some cases, length of life.¹ There is a growing body of evidence linking the modern diet, particularly the balance of dietary fatty acids, to an increased risk of allergic disease. In many industrialized countries, diets are low in Ω -3 (n-3) fatty acids and contain an overabundance of Ω -6 (n-6) fatty acids because of increased consumption of linoleic acid (18:2, n-6)-rich vegetable oils and arachidonic acid (AA, 20:4, n-6) from meat. Diets high in n-6 polyunsaturated fatty acids compete with n-3 long-chain polyunsaturated fatty acids (LCPUFA) for incorporation into cells, giving rise to a predominance of AA in tissues. AA is the substrate required to produce 2-series prostaglandins and 4-series leukotrienes, both of which are highly active mediators of inflammation and play a major role in allergic inflammation.² Conversely, diets high in n-3 LCPUFA (from fatty fish and fish oils) increase cell membrane docosahexaenoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3), inhibiting synthesis of inflammatory AA, which results in a reduction in prostaglandin E synthesis and inhibition of cytokine and immunoglobulin E (IgE) production associated with allergies.³ There is increasing evidence to describe the plausible biological mechanisms of prenatal n-3 LCPUFA supplementation and the potential modulation of the development of IgE-associated allergic disease in offspring with a genetic risk.⁴⁻⁸ Several observational studies have reported protective associations between increased fish intake in pregnancy and allergic disease symptoms in offspring from infancy to 6 years of age.⁹⁻¹⁴ However, these observational studies are unable to establish causality because of the difficulty in adjusting for complex

confounding factors.¹⁵ Randomized controlled trials (RCTs) of n-3 LCPUFA supplementation during pregnancy have reported protective effects; however, apart from 1 16-year registry based follow-up,¹⁶ all reported allergic disease outcomes are in early childhood (between 1 and 3 years of age).^{7,8,17-20} In addition, the small sample size of a number of RCTs^{7,8,19,20} introduces bias and a degree of uncertainty regarding results. A recent systematic review evaluating the overall body of literature on the effects of n-3 LCPUFA intake during pregnancy concludes that further evidence from well-powered, high-quality trials are essential to establish benefits.²¹ Our study was specifically designed to assess the effect of prenatal n-3 LCPUFA supplementation on the incidence of IgE-associated allergic disease at 6 years of age, when respiratory symptoms become more prevalent.

METHODS

This is a 6-year follow-up study of children born to mothers who participated in the double-blind DHA to Optimize Mother Infant Outcome (DOMInO) RCT (Australian New Zealand Clinical Trial Registration Number 12605000569606).²² Full details of the DOMInO trial and entry into the nested allergy cohort have been previously published.^{17,18} In brief, women <21 weeks' gestation with a singleton pregnancy were enrolled during their antenatal clinic visit and randomly allocated to either treatment or control group through a computer-driven telephone randomization service, stratified by center and parity (first birth versus subsequent births). Women were asked to consume capsules containing either 500 mg of fish oil concentrate, providing ~800 mg/day DHA and 100 mg/day EPA (Intervention), or 500 mg vegetable oil capsules from 21 weeks' gestation

until delivery (Incromega 500 TG, Croda Chemicals, East Yorkshire, England). The vegetable oil capsules contained a blend of 3 nongenetically modified oils (grapeseed, sunflower, and palm) in equal proportions. This blend of oils was designed to match the polyunsaturated, monounsaturated, and saturated fatty acid profile of the average Australian diet.²³ All capsules were similar in size, shape, and color, and neither the women nor research staff was aware of the treatment allocated. Women were eligible to enroll in a nested childhood allergy follow-up if their unborn child had a family history of allergic disease (mother, father, or sibling with a history of medically diagnosed eczema, asthma, or hay fever). Before the child's sixth birthday, families who had taken part in an early childhood allergy follow-up^{17,18} were mailed an invitation to take part in a 6-year allergy assessment (Australian New Zealand Clinical Trial Registration Number 12615000498594). Assessments were completed at 2 South Australian centers, the Women's & Children's Hospital and Flinders Medical Centre. Approval for this study was granted by the Women's & Children's Health Network and the Southern Adelaide Clinical Human Research Ethics Committees.

Allergic Disease Outcome Assessments and Definitions

The primary outcome of the 6-year allergy follow-up was the incidence of allergic disease symptoms (eczema, wheeze, rhinitis, rhino-conjunctivitis) with a positive skin prick test (SPT) to ≥ 1 allergen extracts (sensitization) at 6 years of age (referred to as atopic eczema, IgE-associated wheeze, allergic rhinitis, and allergic rhino-conjunctivitis from this point forward).²⁴ Children attended a clinic appointment conducted by 1 of 4 experienced research nurses specifically trained in pediatric SPT procedures. Quality assurance

reviews of the SPT technique were conducted every 6 months by 1 of the investigators (K.P.B.). Sensitization (IgE response) was defined as a positive SPT with a weal size ≥ 3 mm greater than the negative control to at least 1 allergen extract. The allergen extract panel consisted of 3 common foods (hen's egg, peanut, and cashew) and 7 common airborne allergens (ryegrass pollen, olive tree pollen, *Alternaria tenuis*, cat, dog, and 2 species of house dust mite [HDM], *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) (Laboratoire Stallergenes, de Tocqueville, France). Glycerol and histamine phosphate was used for the positive control and the negative control consisted of saline (Hollister-Stier, Spokane, WA). The same research nurses administered the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (for 6–7 year olds) to record symptoms of allergic disease.²⁵ The validated ISAAC questionnaire consists of 3 modules containing symptom based “core” questions used to assess cardinal symptoms and severity of eczema, wheeze, and rhinitis/rhinoconjunctivitis for a period covering the previous 12 months. ISAAC questionnaire modules also include parent-reported disease “labels” or diagnoses (eg, “Has your child ever had asthma?”; “Has your child ever had eczema?”; “Has your child ever had hay fever?”).²⁶ Atopic eczema was defined as the presence of a chronic, itchy rash distributed to the facial, flexural, or extensor surface of the skin with sensitization to at least 1 of the allergens assessed. IgE-associated wheeze was defined as a history of wheezing and/or whistling in the chest within the past 12 months with sensitization to at least 1 of the aeroallergens tested. Allergic rhinitis was defined as a history of sneezing or a runny or blocked nose (in the absence of cold or flu) in the past 12 months along with sensitization to at least

1 of the aeroallergens tested. If symptoms of itchy watery eyes were also present within the past 12 months, this was defined as allergic rhinoconjunctivitis.

If a child was unable to attend a clinic appointment, questionnaires were either posted or e-mailed to the family or administered to the caregiver of the child by telephone. Data were collected on postrandomization characteristics of the child, including schooling, home environment, physical activity, environment, sociodemographic characteristics, and diet. Dietary information collected on the number of fish meals in the last month or the number of fortified foods in the last month was obtained via an interviewer-administered questionnaire.

Sample Size and Statistical Analysis

A total of 706 children were enrolled in the nested allergy follow-up of the DOMInO trial (368 in the n-3 LCPUFA group and 338 in the control group). This sample size provided 95% power to detect a 40% relative reduction in the symptoms of atopic disease at 6 years of age from 30% to 18% ($\alpha = 0.05$, two-sided). It was estimated that 30% of children in the placebo group would have allergies, which was conservative given that this follow-up study includes children with both single and double familial risk. Similarly, the postulated effect size of a 40% relative reduction in allergic disease was modest and realistic compared with the 67% and 59% relative reductions in child allergy outcomes reported by Furuholm et al⁸ and Olsen et al,¹⁶ respectively, in response to fish oil treatment during pregnancy. All analyses were performed according to the intention to treat principle. Multiple imputation, implemented under a missing-at-random assumption and performed separately by randomized group using chained equations,²⁷

was used to address missing outcome data. Imputation models included covariates prespecified for adjustment and auxiliary variables associated with the outcomes. Following analysis of the 100 multiply imputed datasets, individual treatment effect estimates were combined using Rubin's rules.²⁸ The primary analysis was based on imputed data and included all families that consented to participate in the allergy follow-up, excluding missing data due to 2 deaths in the control group and 1 death in the n-3 LCPUFA group. A range of sensitivity analyses were also performed for the primary outcome assuming that data were missing not at random (Supplemental Information). All analyses were adjusted for the stratification variables of center and parity, as well as the baseline characteristic variables of infant gender and maternal history of allergic disease. To address each hypothesis, both unadjusted and adjusted analyses were performed, with the adjusted analyses considered primary. Binary outcomes were analyzed using log binomial regression models, with treatment effects expressed as relative risks (RRs) with 95% confidence intervals (CIs). For each outcome variable, statistical significance was assessed at the 0.05 level using a two-sided comparative test of treatment effect. All analyses were performed by using SAS version 9.3 (SAS Institute, Inc, Cary, NC) and Stata Release 13 (Stata Corp, College Station, TX).

RESULTS

The trial profile of the 6-year allergy follow-up cohort is shown in Fig 1. Following the exclusion of deaths and withdrawals from the original early childhood allergy cohort ($n = 706$), a total of 668 children were eligible and invited to participate in the 6-year follow up. Enrollment began on April 12, 2012, and data collection

was completed on August 29, 2014. A total of 603 of 668 (90.2%) of the available cohort completed a 6-year allergy assessment (85.4% of early childhood allergy cohort), with 485 children undergoing SPT to determine sensitization status.

Participant Characteristics

Characteristics of the families consenting to childhood allergy follow-up were similar between the groups and have been previously reported.¹⁸ In brief, mean maternal age at trial entry was 29.6 years (SD, 5.7 years), and 13.0% of the participating mothers smoked during pregnancy. All unborn children had at least 1 first-degree relative with a history of medically diagnosed allergic disease; there was a history of maternal allergic disease for 70%, paternal disease for 54%, and allergic disease for both parents for 29% of the participants. A total of 39.8% were first-born children (parity 0), and 47.7% were boys. Adverse events, defined as hospitalization >24 hours, were recorded, and there were no differences between the groups (6.4% vs 6.9%). As previously reported,¹⁸ compliance with the intervention was good with 77% of mothers in the n-3 LCPUFA group and 80% of mothers in the control group reporting that they had missed 0 to 3 capsules per week (from a total of 21 capsules per week) at 28 weeks' gestation. DHA concentration in the plasma phospholipids of cord blood from women in the high-DHA group was greater than in the control (median, 7.22% vs 6.09% total phospholipid fatty acids, $P < .001$). Fewer than 2% of mothers in each group chose not to take any capsules. More mothers in the control group (15.2%) versus the n-3 LCPUFA group (9.9%) sought unblinding of their treatment allocation before the 6-year assessment ($P = .03$). Postrandomization characteristics at 6 years of age, including parental education, employment, and

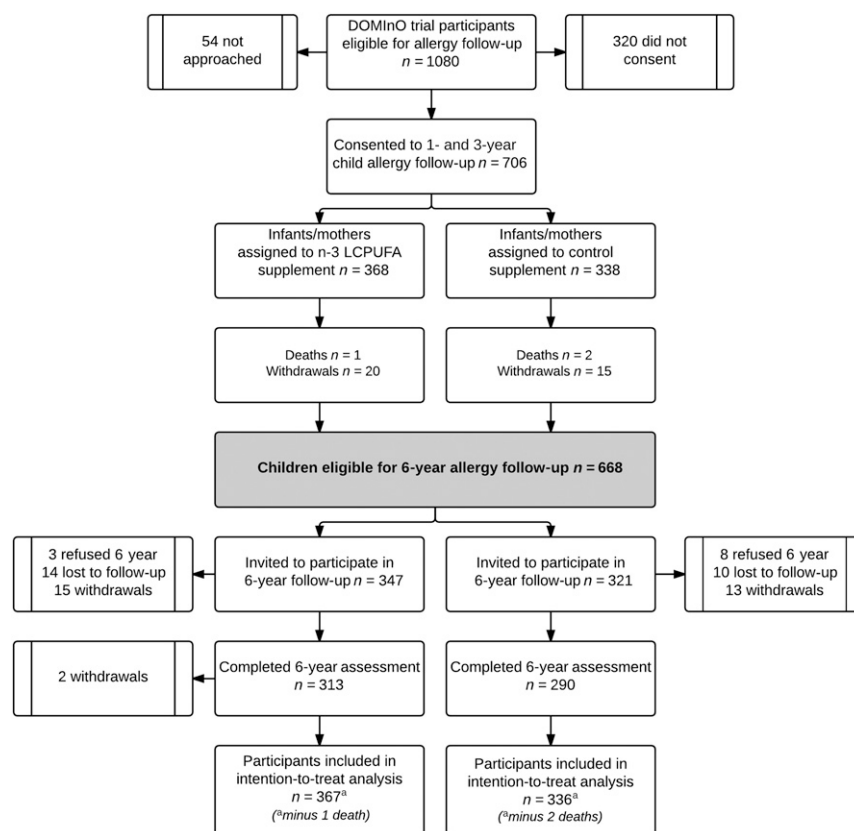


FIGURE 1

Six-year allergy follow-up: trial participation flow diagram. Flow diagram of participant flow through the allergy cohort of the DOMInO trial.

Socioeconomic Index for Area,²⁹ were comparable between the groups (data not presented). Environmental, dietary, and anthropometric characteristics of the child at 6 years of age are shown in Table 1. There were no statistically significant differences between the groups.

Allergic Disease Outcomes

We found no difference in the composite primary outcome of incidence of IgE-associated allergic disease symptoms (eczema, wheeze, or rhinitis), with sensitization at 6 years of age, between the n-3 LCPUFA and control groups (31.48% [116/367] vs 31.46% [106/336]; adjusted relative risk [aRR], 1.04; 95% CI, 0.82–1.33, $P = .73$; Table 2). The overall incidence of “any eczema” at 6 years of age was 15.89%, with 10.27% cases identified as atopic (positive SPT). There was no

difference between the n-3 LCPUFA and control groups in the percentage of children with atopic eczema symptoms (9.90% vs 10.64%; aRR, 0.95; 95% CI, 0.59–1.53; $P = .83$) or parent-report of “Has your child ever had eczema?” (29.42% vs 34.24%; aRR, 0.87; 95% CI, 0.69–1.10; $P = 0.25$; Table 2). The overall incidence of wheeze with sensitization at 6 years of age was 15.0%. There was no difference between the n-3 LCPUFA and control groups in the percentage of children with IgE-associated wheeze (16.36% vs 13.53%; aRR, 1.24; 95% CI, 0.83–1.85; $P = .30$) or parent-report of “Has your child ever had asthma?” (21.41% vs 21.74%; aRR, 1.01; 95% CI, 0.75–1.37; $P = .92$; Table 2). The most common allergic disease symptom at 6 years of age was rhinitis, affecting a total of 31.50% of the participating children. There was no difference in allergic

rhinitis (20.39% vs 21.29%; aRR, 0.98; 95% CI, 0.72–1.35; $P = 0.92$) or allergic rhino-conjunctivitis (12.72% vs 11.49%; aRR, 1.12; 95% CI, 0.72–1.73; $P = .61$) between the n-3 LCPUFA and control groups. There was a nonsignificant 23% reduction in the incidence of parent-report of "Has your child ever had hay fever?" in the n-3 LCPUFA group (22.05% vs 29.05%; aRR, 0.77; 95% CI, 0.59–1.01; $P = 0.06$; Table 2).

Sensitization Outcomes

Analysis of overall allergen sensitization showed no difference between the n-3 LCPUFA and control groups in the percentage of children sensitized to at least 1 allergen extract (50.49% vs 48.61%; aRR, 1.04; 95% CI, 0.90–1.28; $P = .43$; Table 3). There was no difference between the n-3 LCPUFA and control groups on sensitization to individual allergen extracts: hen's egg, peanut, cashew, ryegrass pollen, olive tree pollen, *Alternaria tenuis*, cat, dog, and *D. pteronyssinus*. There was, however, a significant reduction in the percentage of children sensitized to HDM (*D. farina*) in the n-3 LCPUFA group (13.42% vs 20.30%; aRR, 0.67; 95% CI, 0.44–1.00; $P = .0495$; Table 3).

DISCUSSION

This 6-year allergy follow-up of children at hereditary risk of allergic disease was designed to determine the effect of prenatal n-3 LCPUFA supplementation on outcomes of IgE-associated allergic disease in the early school-age child. Our results show that a maternal dose of 900mg of n-3 LCPUFA per day during pregnancy had no effect on the composite primary outcome of eczema, wheeze, rhinitis, or rhino-conjunctivitis with allergen sensitization at 6 years of age. Analyses of secondary outcomes show a significant reduction in sensitization to HDM (*D. farinae*)

TABLE 1 Postrandomization Characteristics of the Child at 6 Years of Age

Characteristic	n-3 LCPUFA ^a	Control ^a	P^b
Child height, cm: mean (SD)	117.9 (5.3)	118.2 (5.4)	.55
Child weight, kg: mean (SD)	22.5 (4.1)	22.7 (3.9)	.67
Child BMI, kg/m ² : median (IQ range)	15.8 (14.9–16.9)	15.9 (14.9–17.2)	.56 ^b
Number of adults in home, (SD)			.68
1	44 (14.0)	47 (16.3)	
2	232 (73.9)	211 (73.0)	
≥3	38 (12.1)	31 (10.7)	
Number other children in home, (SD)			.35
0	44 (14.0)	36 (12.5)	
1	158 (50.3)	130 (45.1)	
2	66 (21.0)	77 (26.7)	
≥3	46 (14.6)	45 (15.6)	
Smoker in household, (SD)			.30
No	217 (69.1)	210 (72.9)	
Yes	97 (30.9)	78 (27.1)	
Cat as pet, (SD)			.76
No	209 (66.6)	189 (65.4)	
Yes	105 (33.4)	100 (34.6)	
Dog as pet, (SD)			.10
No	159 (50.6)	127 (43.9)	
Yes	155 (49.4)	162 (56.1)	
Farm animals, (SD)			.24
No	289 (92.0)	258 (89.3)	
Yes	25 (8.0)	31 (10.7)	
Gas fuel used in home, (SD)			.15
No	69 (22.0)	78 (27.0)	
Yes	245 (78.0)	211 (73.0)	
Dust mite protector mattress or pillow, (SD)			.11
No	223 (71.7)	189 (65.6)	
Yes	88 (28.3)	99 (34.4)	
Number of fish meals in last month: median (IQ range)	4.0 (2.0–5.0)	4.0 (2.0–6.0)	.53 ^b
DHA-fortified foods in last month: median (IQ range)	20.0 (10.0–30.0)	30.0 (12.0–30.0)	.11 ^b
DHA intake via supplements, (SD)			0.85
No	214 (68.4)	199 (69.1)	
Yes	99 (31.6)	89 (30.9)	
Age first received paracetamol: n (%)			.66
≤12 mo	287 (92.9)	257 (91.8)	
>1–<3 y	21 (6.8)	20 (7.1)	
≥3–<5 y	1 (0.3)	2 (0.7)	
≥5 y	0 (0.0)	1 (0.4)	
Paracetamol last 12 mo: n (%)			.36
At least 1/2 wk	4 (1.3)	0 (0.0)	
At least 1/mo	31 (10.0)	29 (10.3)	
At least 1/wk	4 (1.3)	2 (0.7)	
<1/mo	242 (78.1)	227 (80.5)	
0 times in the last 12 mo	29 (9.4)	24 (8.5)	
Age first received ibuprofen: n (%)			.20
≤12 mo	75 (33.9)	77 (42.5)	
>1–<3 y	99 (44.8)	74 (40.9)	
≥3–<5 y	34 (15.4)	18 (9.9)	
≥5 y	13 (5.9)	12 (6.6)	
Ibuprofen last 12 mo: n (%)			.32
At least 1/2 wk	0 (0.0)	1 (0.5)	
At least 1/mo	15 (6.8)	6 (3.3)	
At least 1/wk	3 (1.4)	1 (0.5)	
<1/mo	149 (67.1)	123 (67.2)	
0 times in the last 12 mo	55 (24.8)	52 (28.4)	
Times per week engaging in physical activity: n (%)			.64
Never or occasionally	9 (2.9)	5 (1.7)	

TABLE 1 Continued

Characteristic	n-3 LCPUFA ^a	Control ^a	<i>p</i> ^b
1 or 2 times per week	43 (13.7)	38 (13.2)	
≥3 times per week	262 (83.4)	245 (85.1)	

Denominators based on available data at 6 y IQ, interquartile.

^a For n-3 LCPUFA and control groups, data are number (*n*) of subjects (percentage) unless otherwise indicated.

^b Continuous characteristics compared using independent sample *t* tests or Wilcoxon tests as appropriate, categorical characteristics compared using χ^2 tests.

only. The overall incidence of IgE-associated disease at 6 years of age was higher than that reported in this cohort of children at 1 and 3 years of age^{17,18} and other RCTs of prenatal n-3 LCPUFA supplementation for children at high hereditary risk of allergy^{7,8,20}. This increased incidence is most likely due to the advancing age of the child when assessed and atopic march progression. A total of 31.5% children were identified

as having at least 1 symptom of either atopic eczema, IgE-associated wheeze, or allergic rhinitis/rhino-conjunctivitis. Although more children reported allergic disease symptoms, there appears to be a dilution of the protective effects on sensitization seen in RCTs that assessed symptoms of atopic disease in infancy.^{7,8,18} Our results did not support findings from cohort studies observing an

association with increased maternal dietary n-3 LCPUFA (mainly from fish) intake during pregnancy and reduced incidence of asthma or wheeze^{9,11,12,30} at 5 to 7 years of age and hay fever at 5 years of age.³¹ There was some comparability, however, with 2 studies reporting a reduction in sensitization to HDM at 6 years of age.^{9,12} We used 2 common species of HDM in our SPT allergen extract panel, *D. farinae* and *D. pteronyssinus*, yet there was a significant reduction in sensitization to *D. farinae* only. This inconsistency in results is unexplainable, and the possibility of a chance finding cannot be excluded. Although no significant effects of the intervention was seen in any of the other allergen extracts tested, there was a reduction in sensitization

TABLE 2 Allergic Disease Symptoms at 6 Years of Age

	n-3 LCPUFA <i>n</i> = 367; (%) ^a	Control <i>n</i> = 336; (%) ^a	RR (95% CI)	<i>P</i>	aRR (95% CI) ^b	<i>P</i> ^b
Allergic disease symptoms with sensitization	116 (31.5)	106 (31.5)	1.00 (0.78–1.28)		1.04 (0.82–1.33)	.73
Eczema symptoms with sensitization	36 (9.9)	36 (10.6)	0.93 (0.58–1.50)	.77	0.95 (0.59–1.53)	.83
Wheeze symptoms with sensitization	60 (16.4)	45 (13.5)	1.21 (0.81–1.81)	.35	1.24 (0.83–1.85)	.30
Rhinitis symptoms with sensitization	75 (20.4)	72 (21.3)	0.96 (0.70–1.31)	.79	0.98 (0.72–1.35)	.92
Rhino-conjunctivitis symptoms with sensitization	47 (12.7)	39 (11.5)	1.11 (0.72–1.71)	.64	1.12 (0.72–1.73)	.61
Parent-reported eczema ever	108 (29.4)	115 (34.2)	0.86 (0.68–1.09)	.20	0.87 (0.69–1.10)	.25
Parent-reported asthma ever	79 (21.4)	73 (21.7)	0.98 (0.73–1.34)	.92	1.01 (0.75–1.37)	.93
Parent-reported hayfever ever	81 (22.1)	98 (29.1)	0.76 (0.58–1.00)	.046	0.77 (0.59–1.01)	.055

Data are based on analysis of 100 imputed datasets. RR, relative risk.

^a For n-3 LCPUFA and control groups, data are number of subjects (percentage).

^b Adjusted for enrolling center, parity, child gender, and maternal history of allergic disease.

TABLE 3 Sensitization to Individual Allergen Extracts at 6 Years of Age

Allergen Extract	n-3 LCPUFA <i>n</i> = 367; (%) ^a	Control <i>n</i> = 336; (%) ^a	RR (95% CI)	<i>P</i>	aRR (95% CI) ^b	<i>P</i> ^b
Any	185 (50.5)	163 (48.6)	1.04 (0.87–1.24)	.67	1.07 (0.90–1.28)	.43
Hen's egg ^c	6/248 (2.4)	5/232 (2.2)	1.12 (0.35–3.63)	.85	1.18 (0.37–3.78)	.79
Peanut	27 (7.2)	39 (11.7)	0.62 (0.35–1.09)	.10	0.64 (0.36–1.13)	.13
Cashew ^c	9/249 (3.6)	16/231 (6.9)	0.52 (0.24–1.16)	.11	0.55 (0.25–1.21)	.14
Ryegrass	97 (26.3)	87 (25.9)	1.02 (0.76–1.36)	.92	1.04 (0.78–1.39)	.78
Olive tree pollen	55 (14.9)	41 (12.2)	1.23 (0.77–1.96)	.39	1.27 (0.79–2.03)	.32
<i>D. pteronyssinus</i>	75 (20.4)	83 (24.7)	0.83 (0.59–1.15)	.26	0.84 (0.60–1.17)	.30
<i>D. farinae</i>	49 (13.4)	68 (20.3)	0.66 (0.44–0.99)	.046	0.67 (0.44–1.00)	.049
Cat hair	46 (12.5)	40 (11.9)	1.04 (0.66–1.64)	.85	1.07 (0.68–1.68)	.77
<i>Alternaria tenuis</i>	68 (18.6)	62 (18.4)	1.01 (0.69–1.47)	.96	1.03 (0.71–1.49)	.88
Dog hair	45 (12.3)	40 (12.0)	1.02 (0.63–1.66)	.92	1.07 (0.67–1.72)	.78

Data are based on analysis of 100 imputed datasets unless otherwise indicated. RR, relative risk.

^a For n-3 LCPUFA and control groups, data are number (*n*) of subjects (percentage).

^b Adjusted for enrolling center, parity, child gender, and maternal history of allergic disease.

^c Imputed analyses not done due to rarity of outcomes.

to peanuts and cashews in the n-3 LCPUFA group. Although numbers are low and these findings may be due to chance, this reduction in sensitization to food allergens at 6 years of age is consistent with the reduced incidence of sensitization to food allergens seen at 1 year of age (egg) and may merit further targeted research.

The DOMInO Trial²² is the largest RCT of n-3 LCPUFA supplementation during pregnancy with high retention and completion of follow up at 6 years. As demonstrated by the equanimity in descriptive characteristics of participants at 6 years of age, the integrity of the randomization was maintained. An unexpected finding in our study was the observation that more mothers in the control group versus the n-3 LCPUFA group had sought unblinding (15.2% vs 9.9%) before the 6-year allergy follow-up assessment. Unblinding was performed by an independent data management center to ensure all research staff remained blinded. Because this was a postrandomization variable, it could not be adjusted for in the statistical analyses when estimating treatment effects; however, there was no relationship between blinded and unblinded families who attended assessments or between the postrandomization characteristics of blinded versus unblinded families.

Another potential limitation of our study (previously reported¹⁸) was the observation of a difference between the groups in infant feeding postrandomization. More infants in the n-3 LCPUFA group were initially breastfed (348/362 [96%]) than in the control group (303/333 [91%]). However, three-quarters of the infants were fed cows' milk-based formula before 6 months of age, with exploratory analyses showing no relation between the initiation of breast feeding and early outcomes of allergic disease at 12 months. Analysis of ISAAC environmental questionnaire diet variables³² at 6 years (data not included) showed no difference between the groups in child diet. It is unlikely child diet, including the small imbalance in early infant feeding, would influence the outcomes of the trial.

CONCLUSIONS

This 6-year allergy follow-up study was designed to resolve uncertainties surrounding the use of maternal n-3 LCPUFA supplementation during pregnancy as an allergic disease preventative strategy. Although suggestive of a reduction in sensitization, our results conclude that there is no significant long-term benefit of prenatal n-3 LCPUFA supplementation for the incidence of allergy in offspring with a familial history of disease. One could

question whether supplementation from conception (or preconception) and continuation throughout breastfeeding would augment effects, and this may warrant further investigation.

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ABBREVIATIONS

AA: arachidonic acid
aRR: adjusted relative risk
CI: confidence interval
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
HDM: house dust mite, IgE, immunoglobulin E
ISAAC: International Study of Asthma and Allergies in Childhood
LCPUFA: long-chain polyunsaturated fatty acids
n-3: Ω -3
n-6: Ω -6
RCT: randomized controlled trial
SPT: skin prick test

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Address correspondence to Dr Karen Best, South Australian Health and Medical Research Institute, Level 7, Clarence Reiger Building, 72 King William Rd, North Adelaide 5006, SA, Australia; E-mail: karen.best@adelaide.edu.au

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Karen P. Best, Thomas Sullivan, Debra Palmer, Michael Gold, Declan (John) Kennedy, James Martin and Maria Makrides

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