Received: 9 March 2023 | Revised: 11 July 2023 | Accepted: 25 July 2023

DOI: 10.1111/pai.14004

ORIGINAL ARTICLE

WILEY

Maternal fish oil and/or probiotics intervention: Allergic diseases in children up to two years old

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Funding information
Academy of Finland, Grant/Award Number: 258606; Allergy, Skin and Asthma Federation Finland; Business Finland; Diabetes Research Foundation Finland; Juho Vainion Säätiö; State research funding for university-level health research of the Turku University Hospital Expert Responsibility Area; the Päivikki and Sakari Sohlberg Foundation, Business Finland, Grant/Award Number: 3486/31/2015

Editor: Agnes Sze Yin Leung

Abstract

Background: As n-3 long-chain polyunsaturated fatty acids and probiotics possess immunomodulatory properties, theoretically they could lower the risk of allergic diseases. But their effects remain controversial. We aimed to study the effects of fish oil and probiotics separately or in combination from early pregnancy onwards to lower the risk of allergic diseases in the infants.

Methods: In this double-blind trial, women ($n = 439$) in early pregnancies were randomized into four intervention groups: fish oil + placebo, probiotics + placebo, fish oil + probiotics, and placebo + placebo. Fish oil (1.9 g docosahexaenoic acid and 0.22 g eicosapentaenoic acid) and probiotic (*Lacticaseibacillus rhamnosus* HN001 and *Bifidobacterium animalis* ssp. lactis 420, 10^{10} colony-forming units each) supplements were provided for daily consumption from randomization up to 6 months postpartum. All analyses were adjusted with pet ownership.

Results: No difference between the infants in the four intervention groups were found regarding physician-diagnosed food allergy, atopic eczema, or atopy at the age of 12 or 24 months (all $p > .05$). The probiotic intervention was associated with lower odds of recurrent wheezing at 24 months (OR 0.39, 95% CI 0.18–0.84, $p = .017$), but not at 12 months.

Conclusions: The use of fish oil and/or probiotics from early pregnancy onwards did not lower the odds of childhood allergic diseases or atopy, with the exception of the probiotic intervention which decreased the risk of recurrent wheezing when the infants were two years old. This suggests that the incidence of asthma could also decrease later in childhood and thus these outcomes need to be clarified in further investigations.

KEYWORDS
allergy, atopic eczema, atopy, fish oils, probiotics, wheezing

1 | INTRODUCTION

The immune system of the child is receptive towards environmental stimuli during the fetal period and early childhood. It has been postulated that consumption of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs)[1,2] and probiotic supplements[3] during and after pregnancy could decrease childhood allergic diseases. Immunomodulatory and anti-inflammatory effects are thought to underpin the mechanisms of n-3 LC-PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and probiotics such as specific strains of genus *Lactobacilli* and *Bifidobacteria*.[4] N-3 LC-PUFAs may regulate the incidence of allergic disease by modifying the production of eicosanoids, increasing cell membrane fluidity, and subsequently inhibiting the effects of NF-κB and decreasing the expression of proinflammatory cytokines.[5,6] Probiotics modulate the immune system through colonization of the intestine which leads to alterations in cytokine expression and T cell production.[7] However, there are also clinical studies of these supplements with regard to allergic disease demonstrating null effects.[8,9]

We hypothesized that both fish oil and probiotics would decrease the incidence of allergic diseases in childhood and when combined, their effects could be additive. The aim of this study was to determine the clinical benefits of an intervention with n-3 LC-PUFA (fish oil) and probiotics, in a novel study setting, that is, individually and when combined, in a double-blinded placebo controlled randomized trial, from early pregnancy until 6 months after delivery on the risk of allergic disease in children up to 24 months of age.

Key Message

Allergic diseases represent a rising global burden and necessitate new preventive measures. This is the first study to assess the combined effects of fish oil and probiotic supplements during pregnancy on the risk of allergic diseases in children in early childhood. Our results demonstrated a reduced risk of recurrent wheezing by the age of 24 months after probiotic supplementation during and after pregnancy.

2 | METHODS

2.1 | Study subjects

Recruitment was carried out in University of Turku and Turku University Hospital between 2013 and 2017 (ClinicalTrials.gov, NCT01922791). The primary aims were to investigate the effects of fish oil and/or probiotic supplement on the risk of gestational diabetes mellitus[10] and on preventing allergic diseases in their offspring. The inclusion criteria: self-reported prepregnancy BMI ≥ 25 kg/m^2, less than 18 gestational weeks (gw), and absence of chronic diseases (allergic diseases allowed). The exclusion criteria: multifetal pregnancy, chronic diseases impacting on metabolic and gastrointestinal health including inflammatory bowel diseases, refusal to terminate the intake of other probiotic or fish oil supplements, diagnosis, or history of coagulopathy, and use of anticoagulants. The study complied with the Declaration of Helsinki 2000. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol, and women provided written informed consent. Of 439 women randomized, the current study investigated a total of 287 children, 284 at 12 months and 264 at 24 months of age (Figure 1).

2.2 | Study design

The study was a randomized, double-blinded, placebo-controlled, and one-center trial. The study information was distributed in maternal welfare clinics, media, and social media with the interested women contacted by the project coordinator who provided further information and scheduling of the study visit. The women were randomized consecutively (at mean 14 ± 2 gw) into four groups: fish oil + placebo for probiotics, probiotics + placebo for fish oil, fish oil + probiotics, or placebo for probiotics + placebo for fish oil. Stratified permuted block randomization was performed by a statistician who was not involved in either study recruitment or its execution. The staff responsible for participant enrollment and study visits remained blinded to the intervention, as were the participants. Information was gathered by interviews, questionnaires, and blood samples, collected for allergen-specific IgE analysis, at 3, 6, 12, and 24 months of age. The questionnaires included questions on history of physician-diagnosed allergic diseases of the child, the child's regular medications; a standardized ISAAC questionnaire[11] was also completed by the parents.

2.3 | Intervention supplements

The food supplements were provided to the women from early pregnancy up to 6 months postpartum. The fish oil capsules (2 capsules/day) (Croda Europe Ltd.) contained a total of 2.4 g of n-3 fatty acids, of which 79% (1.9 g) was docosahexaenoic acid (22:6 n-3, DHA) and 9.4% (0.22 g) eicosapentaenoic acid (20:5 n-3, EPA), the rest being other n-3 fatty acids, including docosapentaenoic acid. Placebo capsules for fish oil contained an equal amount of medium-chain fatty acids (capric acid C8 54.6% and caprylic acid C10 40.3%) and were of the same size, shape, color, and lemon flavor as the fish oil capsules.

Probiotic capsules (1 capsule/day) contained *Lacticaseibacillus rhamnosus* HN001 (formerly *Lactobacillus rhamnosus* HN001) (ATCC SD5675; DuPont) and *Bifidobacterium animalis* ssp. lactis 420 (DSM 22089; DuPont), each 10^{10} colony-forming units per capsule. Placebo capsules for the probiotics consisted of microcrystalline cellulose; the capsules were identical to the probiotic capsules in size, shape, and color.

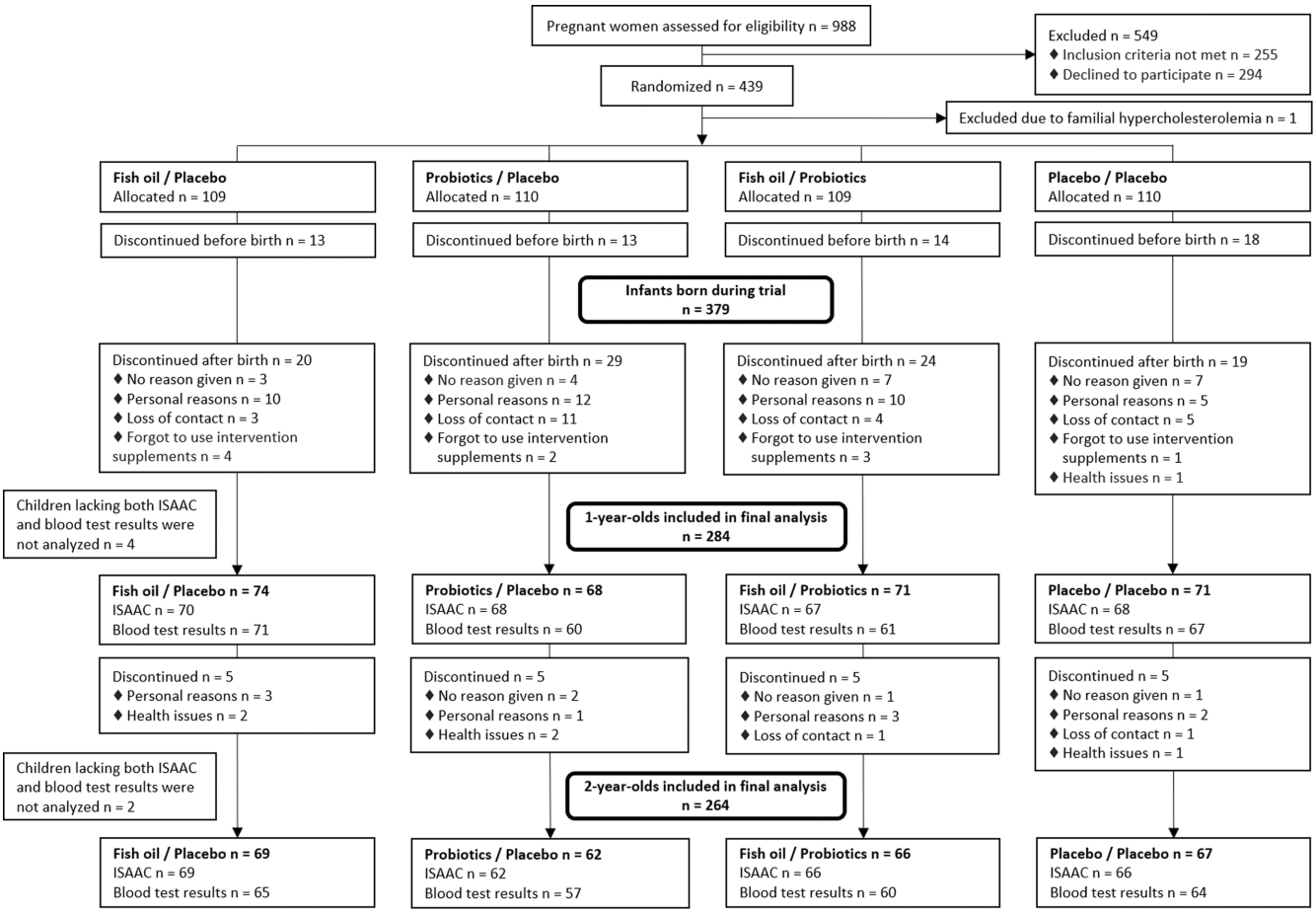

FIGURE 1 Study flow chart.

The compliance with the intervention was reported as good by 88.4% of the women based on interview and when calculated from the returned fish oil capsules, a mean of 91.8% (SD 15.9) of the capsules had been consumed. Good compliance was confirmed in principal component analysis, which revealed a clear separation of the intervention groups according to lipids that reflected the intake of fish oil.[12] The women were asked to keep a diary on a weekly basis to record possible adverse effects. Findings of the adverse effects were minimal.[10]

2.4 | Definition of allergic diseases

Diagnoses of atopic eczema, allergic rhinoconjunctivitis, wheezing, asthma, and food allergy, made by the child's personal physician, were inquired in the questionnaires. Atopic eczema was confirmed by its typical characteristics: morphology, distribution, itching as well as chronic and relapsing course of the disease. Wheezing refers to an expiratory breathing difficulty with a high-pitch sound during expiration. Asthma was diagnosed based on symptoms and lung function tests according to Finnish Current Care Guidelines[13] by personal physician. Recurrence of symptoms was defined by the occurrence of the symptoms within the last 6 months. Atopic sensitization was defined as positive serum IgE antibodies (≥0.35 kU/L) against at least one of the following allergens cat, dog, horse, birch, mugwort, timothy, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, codfish, cow's milk, egg, peanut, soybean, or wheat (Phadiatop Combi®, Phadia). The blood samples were drawn on the morning of the 12- and 24-month visits and analyzed in a certified laboratory (TYKSLAB, the Hospital District of Southwest Finland, Turku, Finland). Sensitization was further subdivided into groups of aeroallergen sensitization and food sensitization. The diagnosis of allergic rhinoconjunctivitis was based on typical symptoms with positive IgE sensitization or a skin prick test and the diagnosis of food allergy based on avoidance-exposure test or positive IgE sensitization with typical symptoms, both diagnoses made by personal physician.

2.5 | Outcomes

The predefined primary outcomes of the study were physician-diagnosed food allergy or atopic eczema at the child's age of 12 and 24 months. The predefined secondary outcomes included the incidence of recurrent wheezing and cough, physician-diagnosed asthma, regular asthma medication, and atopic sensitization.

2.6 | Statistical analysis

The sample size was calculated on the basis of the primary outcome variables (power of 80% and significance level $p < .05$). A 30% prevalence of allergic diseases in child was assumed in the control group and 13% prevalence in the two intervention groups of probiotics + placebo[14] and fish oil + placebo[15] with a further 3% decrease, that is, 10% prevalence in the combined intervention group of fish oil + probiotics. It was calculated that a sample size of 91 per group would be needed to demonstrate a statistically significant difference between the groups.

The characteristics of the children and differences in the study outcomes between the four intervention groups were analyzed using two-sample *t*-test and one-way analysis of variance for normally distributed and Wilcoxon rank-sum test and Kruskal–Wallis test for non-normally distributed continuous variables. Categorical variables were analyzed using Chi-square test, or Fisher's exact test.

Between the four intervention groups, the differences in the incidence of allergic diseases at 12 and 24 months were analyzed with binary logistic regression. In addition, the associations of the main effects of fish oil and probiotics and fish oil × probiotics interaction effect on the allergic diseases were analyzed with the results shown as odds ratios (OR) with 95% confidence intervals (CI). All logistic regression analyses were adjusted with the patients' characteristics "Mother's prepregnancy BMI" and "Owning pets during pregnancy". The logistic regression analysis was not performed when $n \leq 15$ (physician-diagnosed wheezing, regular asthma medication and aeroallergen sensitization at both 12 and 24 months). All *p*-values < .05 were considered significant. Analyses were performed using JMP Pro, version 16.0.0 (SAS Institute Inc., Cary, NC).

3 | RESULTS

3.1 | Study population

The children's characteristics are shown in Table 1. No differences between the four intervention groups were detected. The combined intervention groups differed in prepregnancy BMI when comparing the probiotics group to the no probiotics group and in pet ownership during pregnancy when comparing the fish oil group to the no fish oil group (Table S2), and thus were included as covariates in all the logistic regression analyses.

Physician-diagnosed food allergy was reported in 4.5% and 9.8%, atopic eczema in 15% and 18%, and recurrent wheezing in 12% and 15% of children at 12 and 24 months of age, respectively. (Table 2 for 24-month and Table S3 for 12-month incidence).

3.2 | Impact of the intervention

Fish oil or probiotic intervention did not lower the odds of food allergy or atopic eczema at 12 (all $p > .05$, Table S4) or 24 months (all $p > .05$, Table 3). Furthermore, no difference between the four intervention groups was detected for the odds of developing food allergy or atopic eczema (all $p > .05$, Tables S5 and S6). The results did not differ when comparing reported food allergy to reported food allergy with matching food IgE sensitization (data not shown).

The group receiving probiotics had a significantly lower rate of parent-reported recurrent wheezing when compared to the group who did not receive probiotics; this was associated with a lower odd of recurrent wheezing (adjusted OR 0.39, 95% CI 0.18–0.84, $p = .016$) at the age of 24 months (Figure 2). When the three treatment groups were compared to placebo, the fish oil + probiotics group had a significantly lower odds of recurrent wheezing (adjusted OR 0.33, CI 0.11–0.98, $p = .046$) at 24 months (Table S6). At 12 months, there were no difference between any of the four treatment groups, nor the combined fish oil or combined probiotics groups in terms of the odds of recurrent wheezing (all $p > .05$, Tables S4 and S5).

When evaluating differences between the four study groups, no differences were found for the odds of recurrent cough, asthma, regular asthma medication, and atopic sensitization (all $p > .05$). There were no differences observed in outcome variables when the fish oil receiving group was compared to those who did not receive fish oil and probiotics group compared to the group receiving no probiotics (all $p > .05$, Table 3 and Table S4).

4 | DISCUSSION

To our knowledge, this is the first study to examine whether a combination of fish oil and probiotic supplements during pregnancy affects the risk of allergic diseases in children by the age of 24 months. Our main findings were that (1) fish oil or probiotic intervention during pregnancy did not decrease the risk of physician-diagnosed food allergies or atopic eczema, and combining fish oil with the probiotic intervention yielded no additional effects, (2) the probiotic intervention was connected to a lower risk of recurrent wheezing at the age of 24 months.

When the infants were 24 months old, no differences were found between the groups regarding the development of food allergy, atopic eczema, or atopic sensitization. The findings were in line with some previous intervention studies,[8,16] although meta-analyses have suggested maternal fish oil[2,8] and probiotic[3] intervention could reduce risk of atopic eczema, food sensitization, and atopy. Except for the reduction in wheezing with probiotics, the lack of intervention effect with either fish oil or probiotics in our study, could arise from several reasons. Unlike most previous studies, we did not target high-risk families, although these results have also been reported in the general population.[3,8] The dosage of our fish oil supplements[15] and use of probiotic mixture[17] including *Lacticaseibacillus* genus[18] are in line with previous trials as was the study's duration and do not offer an explanation for the differing results.

Interestingly, we demonstrated that probiotic supplementation during pregnancy was associated with a reduced risk of recurrent wheezing in early childhood. We could not identify studies with a

TABLE 1 Characteristics of the intervention groups.

	Total	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	
	n = 287	*n* = 75	*n* = 69	*n* = 71	*n* = 72	*p*-Value
Mother's self-reported prepregnancy weight, kg, median (Q1, Q3)	80.0 (73.0, 90.0)	83.0 (73.0, 92.0)	78.0 (70.0, 91.0)	79.0 (73.0, 89.0)	80.0 (75.3, 89.0)	.377
Mother's prepregnancy BMI, median (Q1, Q3)	28.7 (26.4, 31.7)	29.4 (27.4, 32.6)	27.6 (26.2, 30.3)	28.3 (26.2, 31.4)	29.3 (26.3, 31.8)	.098
Obese, *n* (%)	111 (39%)	35 (47%)	21 (30%)	26 (37%)	29 (40%)	.241
Mother has a college or university education, *n* (%)	189 (66%)	53 (71%)	47 (68%)	44 (62%)	45 (63%)	.623
Sex, male, *n* (%)	147 (51%)	37 (49%)	37 (54%)	33 (47%)	40 (56%)	.695
Birth weight, g, mean (SD)	3610 (546)	3630 (548)	3610 (555)	3610 (578)	3610 (516)	.997
Gestational weeks at birth, median (Q1, Q3)	40.0 (39.0, 40.7)	40.0 (39.3, 40.6)	40.1 (39.0, 40.7)	40.1 (39.1, 40.7)	39.7 (38.5, 40.5)	.471
Self-reported asthma, *n* (%)						
Mother	41/280 (15%)	9/73 (12%)	11/68 (16%)	9/69 (13%)	12/70 (17%)	.818
Father	39/277 (14%)	7/72 (9.7%)	8/65 (12%)	10/70 (14%)	14/70 (20%)	.344
Mother or father	76/273 (28%)	15/70 (21%)	19/65 (29%)	18/68 (27%)	24/70 (34%)	.391
Self-reported allergy[a], *n* (%)						
Mother	163/287 (57%)	44/75 (59%)	37/69 (54%)	41/71 (58%)	41/72 (57%)	.937
Father	117/280 (42%)	32/73 (44%)	28/66 (42%)	28/70 (40%)	29/71 (41%)	.968
Mother or father	211/281 (75%)	58/73 (80%)	52/67 (78%)	51/70 (73%)	50/71 (70%)	.576
Mother's smoking, *n* (%)						
During pregnancy	8/281 (2.9%)	0/73 (0%)	1/68 (1.5%)	3/68 (4.4%)	4/72 (5.6%)	.126
1-year follow-up	23/261 (8.8%)	5/70 (7.1%)	7/62 (11%)	5/63 (7.9%)	6/66 (9.1%)	.854
2-year follow-up	25/187 (13%)	4/51 (7.8%)	7/52 (13%)	6/44 (14%)	8/40 (20%)	.413
Number of children in family, median (Q1, Q3)	2 (1, 2)	2 (1, 2)	2 (1, 2)	2 (1, 2)	2 (1, 2)	.774
Daycare, *n* (%)						
1-year follow-up						
Home	187/282 (66%)	50/74 (68%)	43/67 (64%)	46/70 (66%)	48/71 (68%)	.998
Home, attending children's clubs 1–2 times per week	68/282 (24%)	17/74 (23%)	17/67 (25%)	16/70 (23%)	18/71 (25%)	
Family-based day care	14/282 (5.0%)	3/74 (4.1%)	4/67 (6.0%)	4/70 (5.7%)	3/71 (4.2%)	
Kindergarten	13/282 (4.6%)	4/74 (5.4%)	3/67 (4.5%)	4/70 (5.7%)	2/71 (2.8%)	
2-year follow-up						
Home	58/245 (24%)	14/61 (23%)	10/58 (17%)	18/63 (29%)	16/63 (25%)	.855
Home, attending children's clubs 1–2 times per week	29/245 (12%)	7/61 (12%)	6/58 (10%)	8/63 (13%)	8/63 (13%)	
Family-based day care	42/245 (17%)	13/61 (21%)	12/58 (21%)	7/63 (11%)	10/63 (16%)	
Kindergarten	116/245 (48%)	27/61 (44%)	30/58 (52%)	30/63 (48%)	29/63 (46%)	

(Continues)

TABLE 1 (Continued)

	Total	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	
	n = 287	n = 75	n = 69	n = 71	n = 72	p-Value
Pets						
During pregnancy	157/283 (56%)	49/72 (68%)	32/69 (46%)	39/71 (55%)	37/71 (52%)	.064
1-year follow-up	136/262 (52%)	44/71 (62%)	26/62 (42%)	31/62 (50%)	35/67 (52%)	.142
2-year follow-up	117/232 (50%)	34/56 (61%)	28/60 (47%)	26/56 (46%)	29/60 (48%)	.366
Duration of exclusive breastfeeding, months, median (Q1, Q3)	0 (0, 3.5)	0 (0, 0.9)	0 (0, 4.0)	0 (0, 4.0)	0 (0, 3.9)	.771
Breastfeeding, months, mean (SD)	11 (6.7)	10 (6.5)	10 (7.4)	11 (6.2)	11 (6.9)	.825

Note: There were no significant differences between the intervention groups except with respect to mother's prepregnancy BMI ($p = .021$) which was higher in the no probiotics group when compared to the probiotics group and having pets at home during the pregnancy ($p = .038$) which was higher in the fish oil group when compared to the no fish oil group (Table S1).

Abbreviations: Q1, Lower quartile; Q3, Upper quartile; SD, Standard deviation.

[a]Including allergic rhinoconjunctivitis and food allergy.

similar setting, that is, initiating already in pregnancy, but a previous placebo-controlled trial conducted in children under 7 months of age with atopic dermatitis lowered their risk of frequent wheezing[19] after supplementation with a symbiotic for 12 weeks. Viral respiratory infections in early childhood have been linked to increased risk of asthma-like symptoms in later childhood in recent studies[20,21] and in previous studies, probiotics have been shown to decrease the respiratory infection rate.[22] This could be the underlying mechanism behind the declining risk of recurrent wheezing after probiotic supplementation, but further research is needed. We did not find a reduced risk of recurrent wheezing separately with the fish oil intervention. Previously, a fish oil intervention provided during and after pregnancy reduced the risk of asthma and/or wheezing[23] in children, but overall conclusions from meta-analyses have not found supporting evidence.[2,8]

When we compared all our three intervention groups to placebo in terms of recurrent wheezing at 24 months, only the combined fish oil + probiotics group differed from placebo. Nonetheless in the subsequent analysis, the fish oil + probiotics group did not differ from the probiotics + placebo group. There were no other indications for an additive effect of combined intervention on any allergic diseases. In previous studies, probiotic supplementation during pregnancy and after delivery have been connected to changes in fecal microbiota,[24] breast milk, and cord blood, for example, affecting IgA[25] and T cell responses, and cytokine expression.[26] Consumption of fish oils, EPA, and DHA, can affect the fetal immune system influencing cell signaling, gene expression, and compete with arachidonic acid to alter Th2 immune responses, such as cytokine expression.[27-29] These findings have been suggested to link probiotics and fish oils to a lower risk of allergic diseases. Although it is possible that there is a joint impact with fish oil and probiotics, we could not detect a synergistic effect of their combination in terms of allergic disease.

The strengths of our study include the study design, that is, prospective double-blinded/placebo-controlled including both fish oil and probiotics separately but also in combination, carried out in one institute with the tests being performed in a single certified laboratory. Unfortunately, the long duration of the study resulted in a notable dropout rate, especially lower educated mothers, and this likely affected the statistical power to detect differences between the intervention groups. The limitations that also require mentioning are not targeting families with a high risk of allergic diseases only, and the recruited mothers were overweight women with high risk of gestational diabetes. Maternal prepregnancy obesity has been associated with higher risk of offspring allergic diseases[30] and although all our analyses were adjusted with the mother's prepregnancy BMI, this could weaken the generalizability of our results. However, due to the recent statistics over 45% of the women giving birth are overweight in Finland.[31]

In conclusion, the use of fish oil and/or probiotic supplements from early pregnancy onwards was not connected with a decreased risk of childhood allergy or atopic eczema, but the probiotic intervention decreased the odds of recurrent wheezing when the child was two years old. This suggests that the incidence of asthma could

TABLE 2 Incidence of allergic diseases at 24 months.

		Intervention groups				Combined intervention groups[a]			
	Total	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	Fish oil	No fish oil	Probiotics	No probiotics
n (%)	*n* = 264	*n* = 69	*n* = 62	*n* = 66	*n* = 67	*n* = 135	*n* = 129	*n* = 128	*n* = 136
Atopic eczema	48/262 (18%)	11/68 (16%)	11/66 (18%)	15/66 (23%)	11/66 (17%)	26/134 (19%)	22/128 (17%)	26/128 (20%)	22/134 (16%)
Physician diagnosed food allergy	25/254 (9.8%)	6/68 (8.8%)	5/61 (8.2%)	7/61 (12%)	7/64 (11%)	13/129 (10%)	12/125 (9.6%)	12/122 (9.8%)	13/132 (9.9%)
Regular asthma medication	6/263 (2.3%)	2/69 (2.9%)	1/62 (1.6%)	0/66 (0%)	3/66 (4.6%)	2/135 (1.5%)	4/128 (3.1%)	1/128 (0.8%)	5/135 (3.7%)
Physician diagnosed wheezing	6/253 (2.4%)	1/68 (1.5%)	0/60 (0%)	3/61 (4.9%)	2/64 (3.1%)	4/129 (3.1%)	2/124 (1.6%)	3/121 (2.5%)	3/132 (2.3%)
Parent reported recurrent wheezing[b]	39/263 (15%)	15/69 (22%)	6/62 (9.7%)	5/66 (7.6%)	13/66 (20%)	20/135 (15%)	19/128 (15%)	11/128 (8.6%)	28/135 (21%)
IgE sensitization	62/246 (25%)	11/65 (17%)	17/57 (30%)	15/60 (25%)	19/64 (30%)	22/125 (21%)	36/121 (30%)	32/117 (27%)	30/129 (23%)
Aeroallergen IgE sensitization	15/246 (6.1%)	1/65 (1.5%)	9/57 (16%)	3/60 (5.0%)	2/62 (3.1%)	4/125 (3.2%)	11/121 (9.1%)	12/117 (10%)	3/129 (2.3%)
Food IgE sensitization	59/246 (24%)	10/65 (15%)	16/57 (28%)	14/60 (23%)	19/64 (30%)	24/125 (19%)	35/121 (29%)	30/117 (26%)	29/129 (23%)
Any allergic disease[c] or atopic eczema	74/263 (28%)	16/69 (23%)	16/62 (26%)	22/66 (33%)	20/66 (30%)	38/135 (28%)	36/128 (28%)	38/128 (30%)	36/135 (27%)

Note: There were no significant differences between the study groups (all $p > .05$), except for parent reported recurrent wheezing.

[a]The combined group 'fish oil' included fish oil + placebo and fish oil + probiotics, and the group 'no fish oil' probiotics + placebo and placebo + placebo. The 'probiotics' group consisted of probiotics + placebo and fish oil + probiotics, and 'no probiotics' of fish oil + placebo and placebo + placebo.

[b]Between the four study groups $p = .048$, Chi-square test; between probiotics vs. no-probiotics $p = .0056$, Chi-square test.

[c]Including physician-diagnosed asthma, wheezing, food and aeroallergen allergy.

TABLE 3 The associations of the fish oil and probiotics with the odds of the child developing allergic diseases at 24 months.

	Fish oil effect[a]		Probiotics effect[b]		Fish oil × probiotics interaction effect
Allergic disease	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	*p*-Value
Atopic eczema	1.22 (0.64, 2.33)	.544	1.21 (0.63, 2.31)	.562	.593
Physician diagnosed food allergy	1.03 (0.44, 2.41)	.955	1.03 (0.44, 2.42)	.949	.394
Parent reported recurrent wheezing	0.86 (0.42, 1.78)	.689	0.39 (0.18, 0.84)	**.016**	.831
IgE sensitization	0.65 (0.36, 1.18)	.153	1.28 (0.71, 2.33)	.408	.527
Food sensitization	0.61 (0.33, 1.12)	.113	1.23 (0.67, 2.26)	.497	.429
Any allergic disease[c] or atopic eczema	1.00 (0.58, 1.74)	.991	1.15 (0.66, 2.00)	.625	.147

Note: All logistic regression analyses were adjusted with the patient characteristic "Owning pets during pregnancy" and "Mother's prepregnancy BMI". Physician-diagnosed wheezing, regular asthma medication and aeroallergen sensitization was not included due to $n \leq 15$. Statistically significant *p*-value is bolded.

Abbreviations: CI, Confidence interval; OR, Odds ratio.

[a]Combined fish oil + placebo and fish oil + probiotics versus combined probiotics + placebo and placebo + placebo.

[b]Combined probiotics + placebo and fish oil + probiotics versus combined fish oil + placebo and placebo + placebo.

[c]Food allergy, physician-diagnosed asthma, recurrent wheezing, food or aeroallergen sensitization.

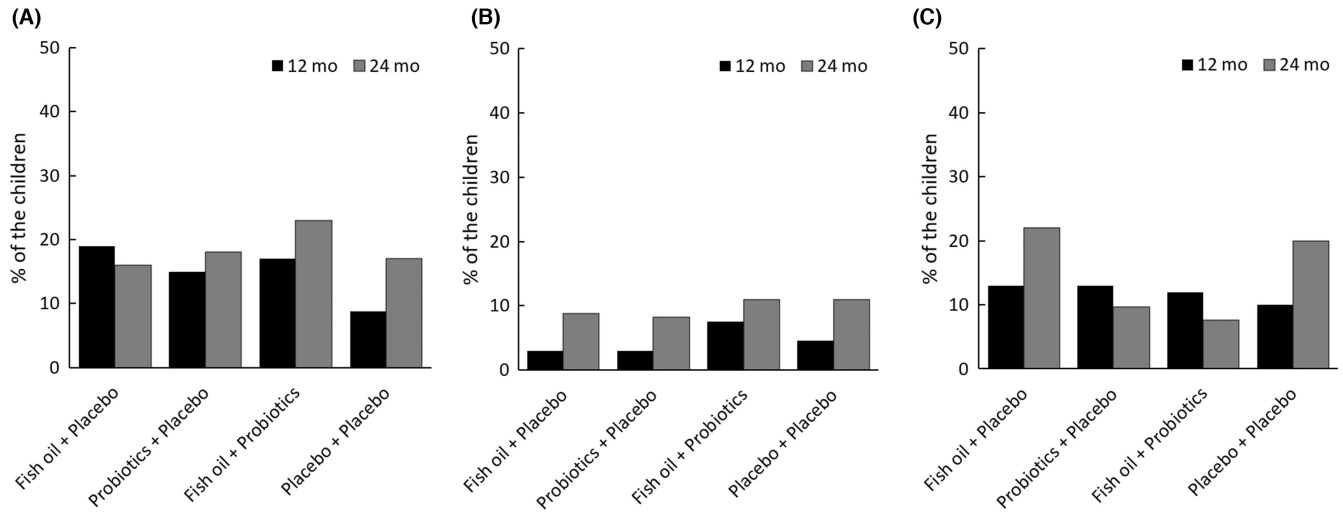

FIGURE 2 Incidence of atopic eczema (A), food allergy (B), and recurrent wheezing (C) in the intervention groups at the age of 12 months (difference between the intervention groups for atopic eczema, food allergy, and recurrent wheezing, respectively, $p = .351$, $p = .648$, and $p = .955$) and 24 months ($p = .753$, $p = .911$, and $p = .048$).

also decrease later in childhood and thus the subject needs further investigation with an extended follow-up period.

AUTHOR CONTRIBUTIONS

Miisa Komulainen: Investigation; writing – original draft; writing – review and editing; visualization; formal analysis; conceptualization. **Lotta Saros:** Investigation; data curation; writing – review and editing. **Tero Vahlberg:** Formal analysis; supervision; writing – review and editing. **Merja Nermes:** Conceptualization; writing – review and editing; methodology. **Tuomas Jartti:** Writing – review and editing; conceptualization; supervision. **Kirsi Laitinen:** Conceptualization; funding acquisition; writing – review and editing; project administration; supervision; methodology; resources.

ACKNOWLEDGMENTS

We thank all of the families that took part in the study and made possible this research.

FUNDING INFORMATION

This work was supported by Academy of Finland (#258606), State research funding for university-level health research of the Turku University Hospital Expert Responsibility Area, the Diabetes Research Foundation, the Juho Vainio Foundation, the Päivikki and Sakari Sohlberg Foundation, Business Finland (3486/31/2015), and Allergy, Skin and Asthma Federation (personal support to M.K.). The probiotic and placebo capsules were provided by DuPont, and fish oil and placebo capsules were provided by Croda Europe Ltd.

The funding sources had no role in the design, execution, analyses, interpretation of the data, or decision to submit results.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest in connection with this paper.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/pai.14004.

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SUPPORTING INFORMATION

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How to cite this article: Komulainen M, Saros L, Vahlberg T, Nermes M, Jartti T, Laitinen K. Maternal fish oil and/or probiotics intervention: Allergic diseases in children up to two years old. *Pediatr Allergy Immunol*. 2023;34:e14004. doi:10.1111/pai.14004