

DHA-Rich Tuna Oil Effectively Suppresses Allergic Symptoms in Mice Allergic to Whey or Peanut^{1–3}

Lieke WJ van den Elsen,^{4,5} Marianne Bol-Schoenmakers,^{4,6} Betty CAM van Esch,^{5,7} Gerard A Hofman,⁵ Bert JM van de Heijning,⁷ Raymond H Pieters,⁶ Joost J Smit,⁶ Johan Garssen,^{5,7} and Linette EM Willemsen^{5*}

⁵Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, ⁶Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands; and ⁷Nutricia Research, Centre for Specialised Nutrition, Utrecht, The Netherlands

Abstract

Background: Supplementation with long-chain n–3 polyunsaturated fatty acids (LCPUFAs) was found to reduce the development of allergic disease.

Objective: The aim was to compare the effectiveness of fish oil diets rich in eicosapentaenoic acid (20:5n–3; EPA) or docosahexaenoic acid (22:6n–3; DHA) to suppress food allergic symptoms.

Methods: Mice were fed a control (10% soybean oil) or fish oil diets rich in EPA (4% soybean oil + 6% EPA oil containing 28.8% EPA and 13.7% DHA) or DHA (4% soybean oil + 6% DHA oil containing 7% EPA and 27.8% DHA), starting 14 d before and for 5 wk during oral sensitization with peanut extract (PE) or whey. Acute allergic skin responses, serum immunoglobulins (Igs), and mucosal mast cell protease-1 (mmcp-1) were assessed. Furthermore, hyperimmune serum was transferred to naive recipient mice fed the different diets.

Results: The DHA diet effectively reduced the acute allergic skin response compared with the control or EPA diet in PE-allergic mice (control, 159 ± 15 , or EPA, 129 ± 8 , vs. DHA, $78 \pm 7 \mu\text{m}$; $P < 0.0001$ or $P < 0.05$, respectively). In contrast, both the DHA and EPA diets reduced the allergic skin response in whey allergic mice (control, 169 ± 9 , vs. DHA, 91 ± 13 , or EPA, $106 \pm 14 \mu\text{m}$; $P < 0.001$ or $P < 0.01$, respectively); however, only the DHA diet reduced mmcp-1 and whey-specific IgE and IgG1. The DHA and EPA diets also reduced the acute skin response in passively immunized mice.

Conclusions: The DHA-rich fish oil reduced allergic sensitization to whey and allergic symptoms in both PE- and whey-allergic mice. These data suggest DHA-rich fish oil is useful as an intervention to prevent or treat food allergic symptoms. *J Nutr* doi: 10.3945/jn.114.198515.

Keywords: food allergy, prevention, n–3 LCPUFA, DHA, EPA

Introduction

The prevalence of food allergies is increasing throughout the world and affects up to 6% of children and 4% of adults (1). Allergic reactions to milk, egg, peanuts, tree nuts, and wheat are increasingly common. These reactions can result in symptoms such as eczema, diarrhea, and hypothermia and in some cases can be life threatening (2). Peanut allergy represents a major

cause of food-induced anaphylaxis (3). Unfortunately, no definitive therapy for food allergy is available yet; therefore, avoidance of the food allergen is the only possibility to prevent clinical manifestations (2).

Peanut allergy generally develops in childhood and is, in 80% of cases, persistent for life (3). In contrast, 90% of children with cow milk allergy outgrow this condition. However, they are still predisposed to other allergic diseases (4, 5). Therefore, the development of novel approaches for the management of allergic disease is of great interest. Dietary components, including n–3 long-chain PUFAs (LCPUFAs)⁸, may be useful in the prevention or treatment of allergic diseases.

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³ Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

⁴ These authors contributed equally to this work.

* To whom correspondence should be addressed. E-mail: l.e.m.willemsen@uu.nl.

⁸ Abbreviations used: AA, arachidonic acid (20:4n–6); LCPUFA, long-chain PUFA; mmcp-1, mucosal mast cell protease 1; PE, peanut extract.

In the same period in which the prevalence of allergic disease increased, dietary habits changed, including an altered consumption of PUFAs (6). The intake of *n*-6 PUFAs, found in margarine and vegetable oils, has increased, whereas the consumption of *n*-3 LCPUFAs, found in oily fish, is traditionally low in westernized countries and has decreased further over the past few decades (6, 7). Among others these dietary changes are suggested to be involved in the increased prevalence of atopy (6, 8, 9). As a consequence, increasing the dietary *n*-3 LCPUFAs content might be a strategy to prevent allergic disease.

Indeed, beneficial effects of fish oil supplementation in the prevention of food allergy were recently found. For example, dietary supplementation with fish oil rich in *n*-3 LCPUFAs during pregnancy and lactation reduced the incidence of food allergy and/or eczema in children at risk of atopy (10–14). Moreover, postnatal *n*-3 LCPUFAs supplementation during the first 6 mo of life reduced allergen-specific type 2 responses (15). Beneficial effects of fish oil supplementation are supported by experimental studies, showing reduced food allergy in mice fed a diet rich in fish oil (16). We have previously shown that a diet rich in DHA suppresses the humoral response and acute allergic symptoms to the cow milk protein whey by enhancing regulatory T cells (17, 18).

n-3 LCPUFAs DHA (22:6*n*-3) and EPA (20:5*n*-3) may function via several mechanisms (19). Dietary lipids can incorporate in the phospholipids of the cell membrane and thus alter functionality of a large variety of cell types (20). EPA serves as a substrate for cyclooxygenases and lipoxygenases, converting EPA into eicosanoids of the 3- and 5-series. These lipidic mediators are considered less proinflammatory than metabolites of the *n*-6 LCPUFAs (20:4*n*-6) arachidonic acid (AA) (21, 22). Besides altering lipid mediator formation, LCPUFAs may act on intracellular and extracellular receptors and transcription factors involved in immune responses (23–26).

In addition to our previous study about the effects of a DHA-rich diet on whey allergy (17), we compared the efficacy of dietary intervention with fish oil high in EPA vs. DHA in the ability to prevent allergic symptoms in mice orally sensitized with peanut extract (PE) or whey and in mice passively immunized with PE or whey hyperimmune serum.

Methods

Diets. Semipurified cow milk protein-free AIN-93G-based diets comprising either 10% soybean oil (control diet), 4% soybean oil plus 6% EPA oil (EPA diet), or 4% soybean oil plus 6% DHA oil (DHA diet) were prepared by Research Diet Services. The extra fat, compared with AIN-93G that contains 7% fat (27), was exchanged for cornstarch as described previously (17). The ratio of *n*-6 to *n*-3 PUFAs was 9.5 for the control diet, 0.7 for the EPA diet, and 1 for the DHA diet. EPA oil was obtained from Equatec. Tuna oil was used as a DHA oil and was a kind gift from Bioriginal, Den Bommel, The Netherlands. The FA composition of these lipid sources is shown in Table 1. The diets were stored at –20°C before use and were refreshed weekly to prevent FA oxidation.

Oral sensitization and challenge of mice. Animal use was performed in accordance with guidelines of the Animal Ethics Committee of Utrecht University (AEC 2010.III.09.108; 2011.III.12.131; 2013.III.01.008). Three-week-old specific pathogen-free female C3H/HeOJ mice (Charles River Laboratories) were fed the control, EPA, or DHA diet, starting 2 wk before the first sensitization and continuing throughout the sensitization period until killing (Supplemental Figure 1). Peanuts were kindly provided by Intersnack Nederland BV, and PE was prepared as previously described (28). Whey protein concentrate 80 (indicated as whey) was obtained from DMV International.

TABLE 1 FA composition of oils used as lipid source for the control, EPA- and DHA-rich diets¹

	Soybean, %FA	EPA, %FA	DHA, %FA
SFA	15.1	14.7	28.9
MUFAs	24.9	20.9	22.8
PUFAs	59.1	54.9	44.5
<i>n</i> -6 PUFAs	53.1	2.8	5.5
18:2 <i>n</i> -6 LA	53.1	1.0	1.3
20:4 <i>n</i> -6 AA	—	1.6	1.8
<i>n</i> -3 PUFAs	5.6	52.1	38.5
18:3 <i>n</i> -3 ALA	5.6	0.8	0.5
20:5 <i>n</i> -3 EPA	—	28.8	7.0
22:5 <i>n</i> -3 DPA	—	3.2	1.4
22:6 <i>n</i> -3 DHA	—	13.7	27.8
Other FA	0.9	9.5	3.8
EPA:DHA	NA	2.1	0.25

¹ AA, arachidonic acid; ALA, α -linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; NA, not applicable.

Mice were sensitized intragastrically by using a blunt needle with 6 mg of PE (8 mice per group and 4 mice for sham sensitization) or 20 mg of whey (6 mice per group and 6 mice for sham sensitization) in 0.5 mL of PBS with 10 μ g of cholera toxin as an adjuvant (List Biological Laboratories Inc), whereas sham mice were administered cholera toxin only (10 μ g/0.5 mL PBS). Mice were orally exposed once a week for 5 consecutive weeks and equipped with an implantable electronic ID transponder (Plexx) on day 28. On day 33 mice were challenged intradermally in the ear pinnae of both ears with 10 μ g of whey or 1 μ g of PE in 20 μ L of PBS to assess the acute allergic skin response as previously described by Schouten et al. (29). Half an hour after the intradermal challenge the anaphylactic shock severity was scored with the scoring table from Li et al. (30), and body temperature was monitored. On the same day, the mice were challenged intragastrically with 15 mg of PE or 50 mg of whey in 0.5 mL of PBS. One and 18 h after the oral challenge blood samples were collected and centrifuged at 14,000g for 15 min. Sera were stored at –70°C until analysis. Mice were killed at day 34 by cervical dislocation.

FA composition erythrocytes. At day 24, blood was collected in heparin tubes, and after centrifugation plasma was removed. Erythrocytes were stored at –70°C until analysis. Erythrocyte lipids were extracted as described by Bligh and Dyer (31) by using C19:0 as an internal standard. The membrane FA composition was assessed by GC as previously described (32).

Serum Igs and mmcp-1. Concentrations of mouse mast cell protease-1 (mmcp-1) were determined in serum collected 1 h after intragastric challenge by means of ELISA (Moredun Scientific Ltd). Concentrations of PE- and whey-specific IgE and IgG1 were determined by ELISA in serum (18 h after intragastric challenge) as previously described (17, 28).

Passive sensitization: transfer of hyperimmune serum to fish oil-fed recipients. To generate hyperimmune sera, mice were immunized intraperitoneally with 100 μ g of PE or whey in alum 2–3 times, after which time blood was collected at day 28. Pooled (hyperimmune) sera from PE- or whey-alum intraperitoneally immunized mice were intravenously transferred (100 μ L) to isoflurane-anesthetized (5% in air) naive mice. These naive mice were fed the control or fish oil diets for 2 wk before injection (*n* = 6 per group). The acute skin response was measured 30 min after serum transfer as described previously.

Histamine-induced acute skin response. Naive mice fed the control diet or fish oil diets for 2 wk (*n* = 6 per group) were administered an intradermal injection in the pinnae of both ears with 20 μ L of saline as a control or 500 μ g of histamine biphosphate (Sigma-Aldrich) in 20 μ L of saline. Ear thickness was measured at basal conditions before injection and 30 min after intradermal treatment.

TABLE 2 Erythrocyte membrane FA composition of PE- or whey-sensitized mice fed the control, EPA, or DHA diet¹

Diet	PE-sensitized, %FA			Whey-sensitized, %FA		
	AA	EPA	DHA	AA	EPA	DHA
Control	14 ± 0.23 ^a	0.099 ± 0.0013 ^c	5.7 ± 0.075 ^c	15 ± 0.091 ^a	0.13 ± 0.021 ^c	6.2 ± 0.067 ^c
EPA	5.4 ± 0.046 ^c	6.8 ± 0.094 ^a	10 ± 0.077 ^b	5.4 ± 0.076 ^c	6.6 ± 0.095 ^a	10 ± 0.058 ^b
DHA	8.3 ± 0.11 ^b	2.5 ± 0.045 ^b	13 ± 0.10 ^a	8.6 ± 0.12 ^b	2.5 ± 0.058 ^b	13 ± 0.16 ^a

¹ Values are means ± SEMs, *n* = 5–8. Means in a column without a common letter are significantly different, *P* < 0.001 for all comparisons (1-factor ANOVA followed by Bonferroni multiple comparison test). AA, arachidonic acid (20:4n-6); PE, peanut extract.

Statistical analyses. Ear swelling data are presented as means ± SEMs and were analyzed with 1-factor ANOVA and post hoc Bonferroni test by using GraphPad Prism software version 5.0 or SPSS version 20. Ig and mmcp-1 data were log-transformed to normalize data distribution and to allow homogenous variance for analysis and data presentation in Tukey box-and-whisker plots. For anaphylactic shock score data Kruskal-Wallis followed by Dunn multiple comparison test was used, and medians are depicted in the graph. Correlations were calculated by using the Spearman correlation test. Differences in body temperature between sham- and whey- or PE-sensitized mice were assessed at several time points by using repeated-measures 2-factor ANOVA (factors: sensitization and time) followed by Bonferroni post hoc test when the interaction term sensitization × time was significant. *P* < 0.05 was considered significant.

Results

EPA and DHA erythrocyte membrane incorporation on fish oil intake. The efficacy of dietary intervention was demonstrated by the increased n-3 LCPUFAs content in erythrocyte membranes in mice fed the EPA or DHA diet, independent of the allergen used for sensitization (Table 2). Incorporation

occurred at the expense of n-6 PUFAs such as AA. Mice fed the DHA diet had substantially increased DHA amounts in their erythrocyte membranes compared with mice fed the EPA and control diets (DHA > EPA > control diet). The EPA membrane content of mice fed the EPA diet was substantially higher than mice fed the DHA or control diet (EPA > DHA > control diet). The AA membrane content of mice fed the control diet was substantially higher than mice fed the EPA or DHA diet; the EPA diet lowered the AA content more than the DHA diet (control > DHA > EPA diet). Dietary intervention did not result in differences in food intake nor in body weight between treatment groups (data not shown).

Acute allergic skin response is reduced in DHA diet-fed whey- or PE-sensitized mice. The acute allergic skin response, as determined by allergen-induced ear swelling, was substantially higher in both PE- or whey-sensitized mice than sham-sensitized mice fed the control diet. In whey-sensitized mice, both the EPA and DHA diets significantly reduced the acute allergic skin response than compared with control diet-fed mice (Figure 1A). In PE-sensitized mice, only the DHA diet

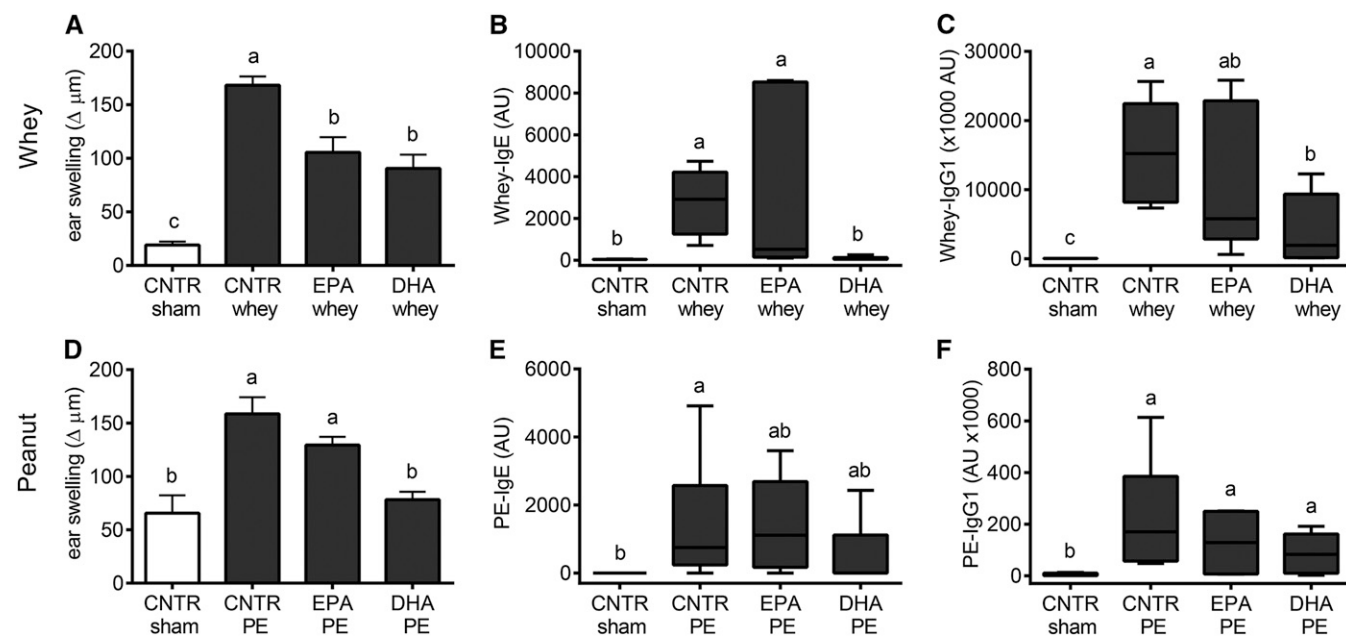


FIGURE 1 The effect of the EPA and DHA diet on the acute allergic skin response and allergic sensitization in whey- or PE-sensitized mice. The acute allergic skin response in (A) whey- or (D) PE-sensitized mice was determined by measuring ear swelling 1 h after intradermal allergen challenge. Furthermore, concentrations of (B) whey-IgE, (C) whey-IgG1, (E) PE-IgE, and (F) PE-IgG1 were determined by ELISA. Data are presented as means ± SEMs (A, D) or Tukey box-and-whisker plots (B, C, E, and F), *n* = 4–8. One-factor ANOVA followed by Bonferroni multiple comparison test (after log-transformation for Ig data). Means/medians without a common letter differ significantly. AU, Arbitrary Unit; CNTR, control; PE, peanut extract.

substantially reduced the allergic skin response. The ear thickness in the DHA-fed mice was similar to that of sham-sensitized mice, and DHA was substantially more effective than the EPA diet (Figure 1D).

DHA diet reduces allergic sensitization in whey-sensitized mice. Whey-specific IgE and IgG1 were enhanced on oral sensitization and reduced in mice fed the DHA diet compared with the control diet. The EPA diet did not reduce whey-IgE or -IgG1, and the DHA diet was more effective than the EPA diet, suppressing whey-IgE to the amount of sham-sensitized mice (Figure 1B, C). PE-specific IgG1 and IgE amounts were enhanced in sensitized mice over mice that received sham-sensitization but not reduced by the diets. PE-IgE, but not PE-IgG1, did not significantly differ between sham mice and PE-sensitized mice fed the fish oil diet (Figure 1E, F). The acute allergic skin response in both whey- and PE-sensitized mice showed a positive correlation with allergen-specific IgE and IgG1, independent of the dietary treatment (Figure 2A–D). The fish oil diets did not affect amounts of total IgE and IgG1 present before sensitization (data not shown).

DHA diet reduces serum mmcp-1 in whey-sensitized mice.

In contrast to the PE model in which body temperature was comparable between PE- and sham-sensitized mice after PE challenge, intradermal whey challenge decreased body temperature of whey- compared with sham-sensitized mice after 30 and 60 min (Figure 3A, B) and increased the anaphylactic symptom score in whey-sensitized mice (Figure 3D). One mouse in the whey-sensitized group fed the control diet died as a consequence of anaphylactic shock. Body temperature and anaphylactic shock severity were not significantly affected by the DHA or EPA diets compared with whey-sensitized mice fed the control diet (Figure 3C, D).

Serum concentrations of mmcp-1, used as a measure of mucosal mast cell degranulation in the intestine, were not substantially higher in either PE- or whey-sensitized mice fed the control diet than sham-sensitized mice. However, in whey-sensitized mice fed the DHA diet mmcp-1 was significantly reduced compared with mice fed the control diet, which positively correlated with the acute allergic skin response, reflecting degranulation of effector cells (Figure 3E, G). The diets did not affect mmcp-1 in PE-sensitized mice, and in these mice no correlation was observed with the acute allergic skin response (Figure 3F, H).

n-3 LCPUFAs reduce the local effector response in naive mice transferred with hyperimmune serum. Mice injected with hyperimmune serum and challenged with the corresponding allergen displayed a significantly higher acute allergic skin response than mice injected with naive serum (Figure 4A, B). The acute allergic skin response was substantially diminished in recipient mice fed the n-3 LCPUFAs diet compared with recipient mice fed the control diet for both PE and whey. The DHA diet was more effective than the EPA diet when whey was used as an allergen. For both PE and whey allergens the ear swelling of mice fed the DHA diet was comparable with the sham control. To reveal whether n-3 PUFAs indirectly suppressed edema formation via an effect on the surrounding tissues, histamine-induced ear swelling was assessed. The acute skin response induced by histamine injection was not altered by the different diets (Figure 4C).

Discussion

In this study, we demonstrate that fish oil rich in DHA suppresses sensitization to whey and allergic symptoms in both whey- and PE-allergic mice.

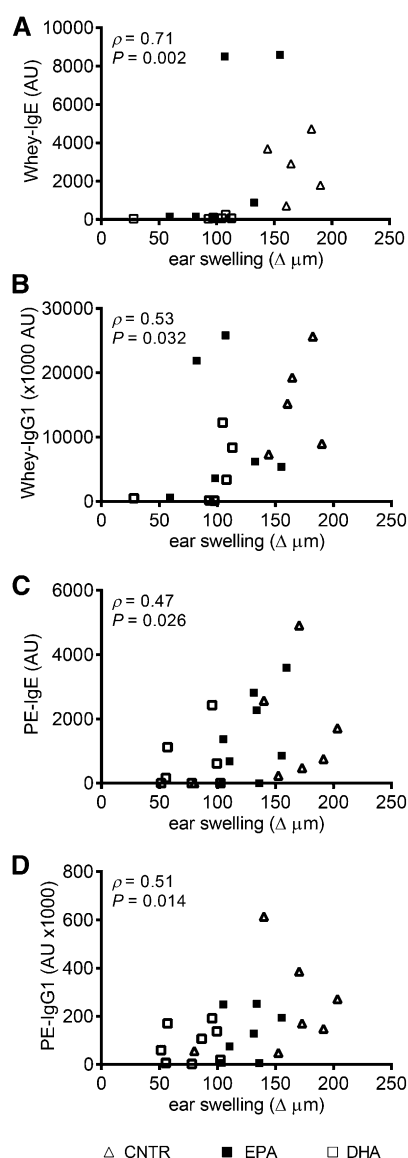


FIGURE 2 Spearman correlation for acute allergic skin response in sensitized mice plotted against allergen-specific Ig (A) whey-IgE, (B) whey-IgG1, (C) PE-IgE, and (D) PE-IgG1, independent of the fed diet ($n = 5$ –8 mice). AU, Arbitrary Unit; CNTR, control; PE, peanut extract.

These results are in concordance with our previous study in which tuna oil rich in DHA largely prevented allergic sensitization to whey in mice (17). Tuna oil contains more DHA than EPA, and in vitro DHA was more effective than EPA in the suppression of Th2-type cytokine secretion by mast cells (33).

Inuit communities have a low prevalence of allergic diseases, presumably associated with their high n-3 LCPUFAs consumption with an n-3:n-6 PUFAs ratio of 2.5:1 (34, 35). In our murine study, the n-3:n-6 intake ratio of 1.4:1 (EPA diet) and 1:1 (DHA diet) is relatively low compared with the ratio in the Inuit diet; however, we interpret this as the highest ratio that realistically could be achieved in humans by dietary intervention with the use of fish oil soft gels. The EPA diet, which contained more n-3 LCPUFAs than the DHA diet, displayed the greatest reduction in membrane AA content, whereas the DHA diet reduced allergic sensitization and/or symptoms most effectively. This suggests that the amount of dietary DHA is more important

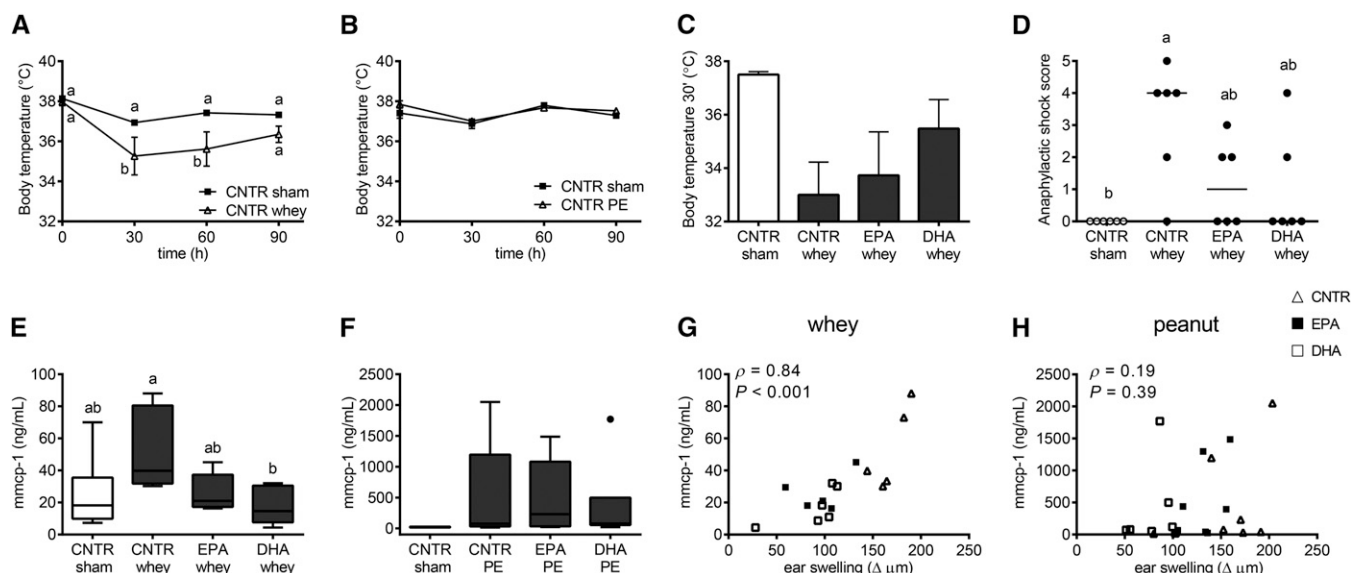


FIGURE 3 The allergic effector response evaluated in whey- or PE-sensitized mice. Body temperature over time after challenge for (A) whey- or (B) PE-sensitized mice fed the control diet. (C) Body temperature and (D) anaphylactic shock symptom scores in whey-sensitized mice fed the different diets at 30 min. Concentrations of serum mmcp-1 in (E) whey- or (F) PE-sensitized mice. Spearman correlations were calculated for ear swelling and mmcp-1 in (G) whey-sensitized and (H) PE-sensitized mice, independent of the fed diet. Data are presented as means $\pm$ SEMs (A–C), Tukey box-and-whisker plots (E, F, outlier indicated as dot), $n = 4$ –8. Repeated-measures 2-factor ANOVA for factors of sensitization and time was used to analyze body temperature curves. Main effects for sham vs. whey (A): sensitization $P = 0.031$, time $P = 0.0001$, sensitization $\times$ time $P = 0.025$; b indicates significant difference in temperature between sham vs. whey sensitization, $P < 0.05$ with Bonferroni post hoc test; for sham vs. peanut (B): sensitization $P = 0.30$, time $P = 0.0001$, sensitization $\times$ time $P = 0.28$. Kruskal-Wallis followed by Dunn multiple comparison test was used for anaphylactic shock data. For other data 1-factor ANOVA between dietary groups followed by Bonferroni multiple comparison test (after log-transformation for mmcp-1) was used. Means/medians without a common letter differ significantly. CNTR, control; mmcp-1, mouse mast cell protease-1; PE, peanut extract.

for its effectiveness in reducing allergy than the membrane content of total n-3 LCPUFAs or n-6 LCPUFAs.

In whey-allergic mice, specific IgE and IgG1 were effectively suppressed by the DHA diet but not the EPA diet. Previously, we also demonstrated that a diet high in DHA suppressed the induction of whey-specific IgE and IgG1 (17). The effects of dietary fish oil supplementation on allergic sensitization differ between studies. In mice fed a 10% fish oil diet serum ovalbumin-specific IgE concentrations tended to increase compared with mice fed a diet rich in sunflower oil (36). However, in another study with a different experimental design, reduced ovalbumin-specific IgE and IgG1 amounts were reported with a 7% fish oil diet (16). In our study the DHA diet effectively suppressed allergic sensitization for whey, but it lacked efficacy in suppressing PE-IgE and -IgG1. However, in these

PE-sensitized mice the reduction in ear swelling correlated positively with PE-IgE and -IgG1. It is possible that the intrinsic ability of PE to act as a strong allergen (37) prevents EPA and DHA from effectively suppressing allergic sensitization. Hence, the effectiveness of n-3 LCPUFAs on the allergic sensitization may depend on the nature of the food allergens.

In whey-sensitized mice the acute allergic skin response (edema formation) and mucosal mast cell degranulation, as determined by serum mmcp-1 (38), were reduced by the DHA diet. These variables were positively correlated, indicating that the DHA diet reduced mast cell degranulation (in the ear and intestine) in whey-sensitized mice. In contrast to whey, PE-sensitized mice did not show a correlation between the acute allergic skin response and mucosal mast cell degranulation. However, in this particular study mmcp-1 in PE-sensitized mice

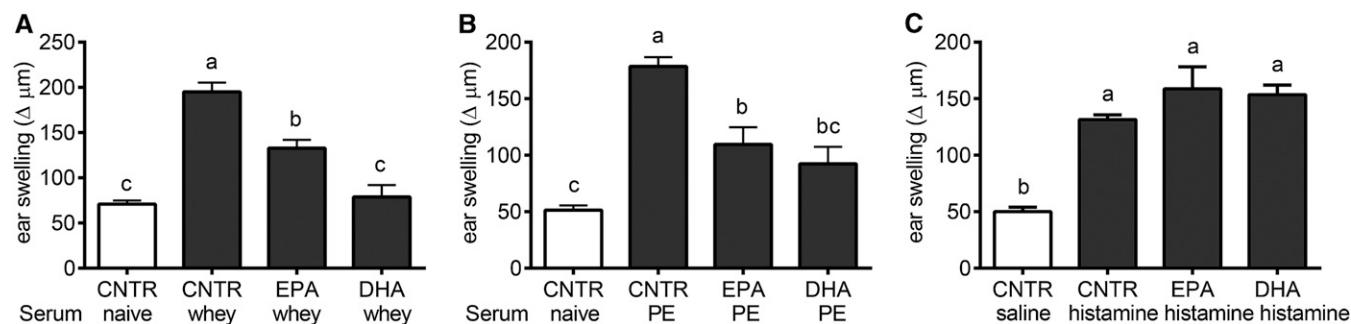


FIGURE 4 The effect of dietary EPA or DHA on the local effector response in passively immunized mice. Acute allergic skin response in mice fed different diets and passively immunized with (A) whey- or (B) PE-hyperimmune sera or (C) mice injected in the ear with histamine. Data are presented as means $\pm$ SEMs, $n = 6$. One-factor ANOVA followed by Bonferroni multiple comparison test. Means without a common letter differ significantly. CNTR, control; PE, peanut extract.

showed a large variance and lack of substantial difference between the sham- and PE-sensitized mice.

To determine the direct effects of n-3 LCPUFAs on the local effector response, beyond their effects on the humoral response, naive recipient mice were fed the EPA or DHA diet and passively transferred with hyperimmune serum after which the acute skin response was determined. The ear swelling response depends on mast cell degranulation (e.g., release of preformed mediators, including histamine), eicosanoids formation (e.g., prostaglandin D₂ production) (39) and the micro milieu of the mast cell [e.g., vascular permeability (40) and interaction with nerve endings (41)]. Most studies suggest that n-3 LCPUFAs do not reduce mast cell histamine release (42, 43). We have also demonstrated this in vitro; however, we found that n-3 LCPUFAs may alter the type of mediators secreted (33). Transfer of hyperimmune serum to naive mice fed the fish oil diets confirmed that n-3 LCPUFAs suppress the local effector response for both food allergens independent of any possible effects on the adaptive immune response. This may be the result of the reduced formation of eicosanoids of the 2/4 series involved in vasodilation and therefore edema formation in the ears (39). This suggests that in addition to inhibition of IgE-mediated effector responses on allergen cross-linking, the EPA and/or DHA diet may also be capable of lowering mast cell-dependent effector responses elicited by generic triggers. The acute skin response after histamine injection remained unaffected, strengthening the hypothesis that n-3 LCPUFAs primarily affect mast cell function and not the sensitivity of surrounding cells or tissue to histamine or other mast cell-derived mediators required to induce symptoms.

Although the EPA-rich diet contained more n-3 LCPUFAs and the AA content of erythrocyte membranes in EPA-fed mice was lower, the DHA-diet reduced IgE and/or allergic symptoms more effectively. Currently, it is unclear why the DHA oil is more effective than the EPA oil. The EPA and DHA oil diets were not selective for either EPA or DHA but contained different ratios of EPA to DHA. This is reflected by different EPA:DHA ratios incorporated in the membranes, being ~3:5 for the EPA diet and 1:5 for the DHA diet. Therefore, it may not only be the absolute amount of DHA present but also its ratio with EPA that contributes to the inhibitory effects observed. The molecular structure of the n-3 LCPUFAs may contribute to a functional difference too. Tomobe et al. (44) showed that dietary intervention with DHA- but not EPA-ethyl ester was capable of reducing the ear swelling response in a mouse model for contact hypersensitivity. DHA is more unsaturated than EPA; thus, it may have enhanced effects on membrane fluidity and lipid raft clustering (45–48). Furthermore, the n-3 LCPUFAs receptor G protein-coupled receptor 120 binds DHA more potently than EPA (26). We have shown in vitro that DHA is the most effective n-3 LCPUFAs in reducing reactive oxygen species and IL-13 secretion by mast cells (33). Together these phenomena may have contributed to a more efficient suppression of the allergic response by the DHA diet over the EPA diet. However, Mickleborough et al. (49) showed EPA to be more effective than DHA in suppressing proinflammatory mediator release from LPS-activated alveolar macrophages from humans with asthma. Hence, the type of target cell as well as the inflammatory trigger involved may be of overall importance in the outcome of the biological effect when comparing EPA with DHA.

In conclusion, in this study we demonstrated that fish oil rich in n-3 LCPUFAs effectively reduced the acute allergic skin response. In addition, DHA-rich fish oil was more effective than EPA-rich fish oil in reducing whey-specific IgE in whey-sensitized mice, the local PE-induced acute skin response in

PE-sensitized mice, and the whey-induced acute skin response in passively immunized mice. Together this suggests that the DHA diet is more effective in reducing allergic symptoms than the EPA-rich diet. The effects were at least partially due to a direct effect on mast cells. These findings could be useful for future interventions intended to prevent or treat food allergy symptoms.

Acknowledgments

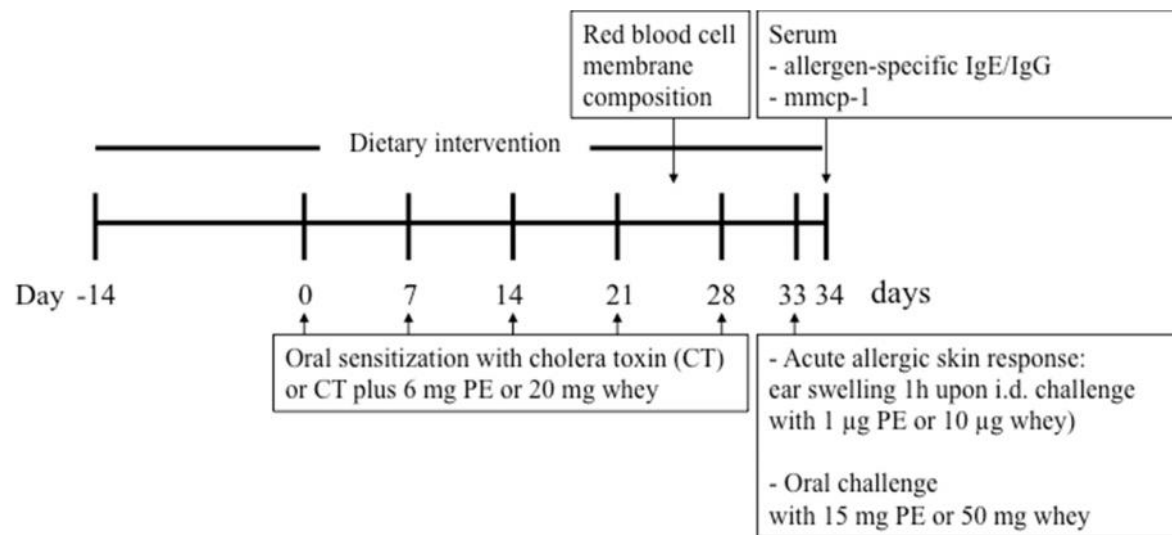
LWJvdE, MB-S, RHP, JJS, and LEMW designed the research; LWJvdE and BJMvdH designed the diets; LWJvdE, MB-S, BCAMvE, and GAH conducted the research; LWJvdE, MB-S, and LEMW analyzed the data; JG provided supervision; LWJvdE, MB-S, and LEMW wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

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Online Supporting Material



Supplemental Figure 1 Schematic overview of the sensitization and challenge protocol and the parameters analyzed in the PE or cow's milk (whey) allergic mice. Mice were fed control, EPA or DHA diet 2 wk prior to and during i.g. sensitization with CT, CT+whey or CT+PE for 5 wk. Mice were sensitized once a wk and one wk after the last sensitization the acute allergic skin response was measured by i.d. allergen challenge in the ear and an oral challenge was provided. Blood samples were collected during the sensitization phase (to determine red blood cell FA incorporation) and 1 h and 18 h (time of killing) after oral challenge to determine mmcp-1 and antigen specific immunoglobulins respectively. CNTR, control; CT, cholera toxin; FA, fatty acid; i.d., intradermal; mmcp-1, mouse mast cell protease-1; PE, peanut extract.