

Dietary Fatty Acids Affect the Immune System in Male Mice Sensitized to Ovalbumin or Vaccinated with Influenza^{1,2}

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Abstract

PUFA are precursor molecules for eicosanoids such as leukotrienes and prostaglandins and may influence immune function through other mechanisms involving membranes, cell signaling, and gene expression. Immune-modulating properties of diets containing different oils [sunflower oil, rich in linoleic acid; linseed oil, rich in α -linolenic acid; salmon oil, rich in marine (n-3) PUFA; and beef tallow, rich in SFA] were investigated in an influenza-vaccination model, in which the delayed-type hypersensitivity (DTH) response was studied in C57BL/6 mice, and an ovalbumin (OVA)-sensitization model for experimental allergy in BALB/c mice. Six-week-old mice were fed the different diets for 7 wk. The first vaccination or OVA sensitization was given 2 wk after the start of the dietary intervention. In the mice vaccinated with influenza, the DTH response to the vaccine was significantly higher in mice fed the marine (n-3) PUFA diet compared to all other groups, indicating that these PUFA promote a T helper-1 response. In the OVA-sensitized mice, those fed the marine (n-3) PUFA diet had a less severe acute allergic skin response (ASR), suggesting that (n-3) PUFA lessen the T helper-2 response. Mice fed the SFA-rich diet had the most severe ASR, indicating that a diet with high levels of SFA may contribute to increased severity of allergic symptoms. Whereas significant differences in *in vivo* immune responses were measured, *in vitro* responses did not differ among the dietary groups. In conclusion, using 2 different models of immune responses demonstrates potential benefits from marine (n-3) PUFA. J. Nutr. doi: 10.3945/jn.110.135863.

Introduction

There are numerous studies investigating the effect of different dietary fatty acids on immune cell functions (1–3). Many animal studies have identified immunomodulatory effects of fish oil, which contains the long-chain (n-3) PUFA (lcPUFA)⁷ EPA and DHA (4). In addition, effects of the plant (n-6) PUFA linoleic acid (LA) and the plant (n-3) PUFA α -linolenic acid (ALA) have been reported, although these have been studied less extensively than EPA and DHA. The mechanisms by which lcPUFA affect immune cells are varied and include altered membrane fluidity, membrane transport processes, raft assembly, intracellular signaling processes, gene expression, and lipid mediator generation (5–9).

It is generally considered that (n-6) and (n-3) PUFA will have different effects on the immune response, because eicosanoid mediators generated from the (n-6) arachidonic acid (AA) are proinflammatory immune regulators (5,10) and (n-3) PUFA act to inhibit production of these mediators (11,12). Prostaglandin (PG) E₂ produced from AA inhibits the function of T helper-1 lymphocytes involved in host defense against bacteria and viruses (13,14) and promotes the function of proallergic T helper-2 lymphocytes (15). Because the combination of EPA and DHA decreases PGE₂ production (16–18), it is expected that these fatty acids will act to enhance T helper-1 responses and host defense and diminish T helper-2 responses and allergic reactions. However, despite the quite extensive literature in this area, there is still no clear consensus on the effects of specific dietary fatty acids on the immune response. This is partly because of inconsistent findings from different studies due to differences in dose of fatty acid used, duration of feeding, animal strain, control diet, and cell responses investigated. Furthermore, most studies have evaluated the effect of dietary fatty acids on functional responses of immune cells measured *ex vivo*. A more robust approach would be to evaluate integrated immune responses *in vivo* (19). Several animal models are available to enable this. These include an ovalbumin (OVA)-induced model of allergic

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⁷ Abbreviations used: AA, arachidonic acid; ALA, α -linolenic acid; ASR, acute allergic skin response; BT, diet enriched in beef tallow; CT, control diet; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; LA, linoleic acid; lcPUFA, long-chain PUFA; LI, diet enriched in linseed oil; PG, prostaglandin; OVA, ovalbumin; SA, diet enriched in salmon oil; s.c., subcutaneous; SO, diet enriched in soy oil; SU, diet enriched in sunflower oil.

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responses in BALB/c mice sensitized to OVA (20) and a vaccine-induced delayed-type hypersensitivity (DTH) model in previously vaccinated C57BL/6 mice (21). Such models have not been widely used to examine the effect of dietary fatty acids on the immune responses. Therefore, we compared the effects of diets containing different amounts of SFA, LA, ALA, and EPA+DHA in these 2 models.

Materials and Methods

Chemicals. OVA (grade V), urethane, β -mercaptoethanol, and o-phenylenediamine dihydrochloride were obtained from Sigma. Aluminum hydroxide was from Pierce. PBS was from BioWhittaker. Tween20 was from Merck and rat-anti-mouse-IgG1-biotin, rat-anti-mouse-IgG2a-biotin, and rat-anti-mouse IgE were from BD Pharmingen. Streptavidin-HRP was from Biosource. Anti-DIG-Fab fragments-HRP was from Roche Diagnostics. RMPI-1640 medium was from Lonza. Anti-mouse CD3mAb (clone 17A2rIgG2b) was purified from a hybridoma obtained from the American Type Tissue Collection. Fetal calf serum (FCS), penicillin, and streptomycin were from Life Technologies. All flow cytometry antibodies were from BD Biosciences, except for F4/80-FITC mAb, which was from AbD Serotec. Anti-FoxP3-APC, Fix / perm-, and permeabilization buffer were from EBioscience and Influvac 2005/2006 was purchased from Solvay Pharma.

Mice. Six-week-old male BALB/c and C57BL/6 mice were obtained from Harlan and housed at constant temperature (20°C) and humidity (40–60%) on a 12-h-light/-dark cycle in the animal facility of Utrecht University. On arrival, mice were offered 1 of the 6 experimental diets. In both experiments, each dietary group consisted of 6 mice. The following designations were used for the different dietary groups; CT: mice fed the control diet; SO, SU, SA, LI, and BT: mice fed diets enriched in soy oil, sunflower oil, salmon oil, linseed oil, and beef tallow, respectively. After 2 wk of consuming the diets, BALB/c mice were sensitized with OVA and C57BL/6 mice were vaccinated with influenza (Fig. 1). Animal care and use were performed in accordance with the guidelines of the Dutch Committee of Animal Experiments and the local university ethical committee.

OVA-induced allergy model. BALB/c mice were sensitized to OVA at 8 and 9 wk of age by i.p. injection of 10 μ g OVA adsorbed into 22.5 mg aluminum hydroxide in 100 μ L saline. One day before the first sensitization, a blood sample was taken by submandibular puncture (20). Acute allergic skin response (ASR) was measured at 11 wk of age by subcutaneous (s.c.) injection of 1 μ g OVA in 25 μ L PBS into the pinna of one ear. The other ear pinna was injected with 25 μ L PBS. Ear thickness, as a readout for a Th2-mediated allergic reaction, was measured in duplicate before antigen challenge and 1 h afterwards by using a digital micrometer (Mitutoyo Digimatic 293561). The ASR was calculated by subtracting the basal ear thickness from the value at 1 h after challenge, correcting for the ear swelling that occurred in the PBS injected ear. At the age of 13 wk, mice were euthanized by i.p. injection of 1 mL 10% urethane and blood was collected via cardiac puncture.

Influenza vaccination model. C57BL/6 mice were vaccinated at 8 and 10 wk of age by s.c. injection of 100 μ L Influvac 2005/2006, diluted 1:50

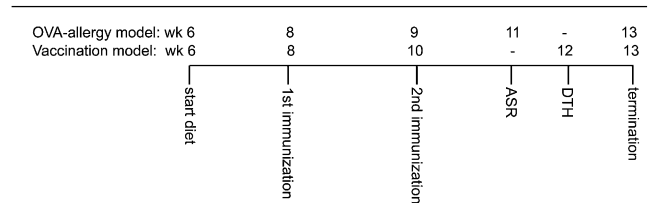


FIGURE 1 Overview of the OVA-allergy model and the influenza-vaccination model. All mice were immunized for the first time at 8 wk of age; the second immunization was at 9 (OVA) or 10 (influenza) wk. Ear-swelling responses were measured at 11 (OVA) or 12 (influenza) wk. Mice were killed at 13 wk of age.

in physiological salt solution, and mixed 1:1 with Stimune (Prionics), which was used as an adjuvant. One day before the first vaccination, a blood sample was taken by submandibular puncture (21). DTH responses were induced at the age of 12 wk by s.c. injection of 25 μ L Influvac into the pinna of one ear, and 25 μ L of PBS was injected into the other ear. Ear thickness, as a readout for Th1-dependent cellular immunity, was measured in duplicate before antigen challenge and 24 h afterwards by with a digital micrometer (Mitutoyo Digimatic). The DTH response was calculated by subtracting the basal ear thickness from the value at 24 h after challenge, correcting for the ear swelling that occurred as a result of s.c. injection of PBS. At the age of 13 wk, mice were euthanized by i.p. injection of 1 mL 10% urethane. Blood was collected via cardiac puncture.

Diets. AIN93M was used as the control diet (22). All experimental diets were AIN93M based and were from Special Diet Services (Dietex International). These experimental diets were enriched with 100 g·kg⁻¹ of each specific oil of interest and consisted of 200 g·kg⁻¹ casein, 360 g·kg⁻¹ cornstarch, 120 g·kg⁻¹ maltodextrin, 90 g·kg⁻¹ dextrose, 50 g·kg⁻¹ Vitacel L600–20 (cellulose fiber), 50 g·kg⁻¹ mixture of vitamins and minerals, and 130 g·kg⁻¹ fat. The fatty acid composition of the different diets is listed in Table 1.

Erythrocyte fatty acids and plasma antibodies. Fatty acid composition of erythrocytes was analyzed as described earlier (23). Influvac- and OVA-specific Ig were measured as previously described (20,21).

Ex vivo cytokine production by splenocytes. Preparation and incubation of splenocytes for the analysis of ex vivo cytokine production was carried out as described earlier (23). Cytokine concentrations in supernatants were analyzed by ELISA conducted according to the manufacturer’s instructions (PharMingen). Concentrations of IFN γ and IL-4, IL-5, and IL-10 were measured. The lower detection limits of the ELISA were 6 ng/L for IL-4, 16 ng/L for IFN γ , 32 ng/L for IL-5, and 10 ng/L for IL-10.

Spleen, thymus, and bone marrow cell subsets. Splenocyte, thymocyte, and bone marrow cell suspensions were prepared as described above for splenocytes and, unless indicated otherwise, all incubations were performed at room temperature in the dark. 10⁵ cells were blocked in PBS, 1% BSA, 5% FCS for 30 min. Cells were collected

TABLE 1 Fatty acid composition of the different experimental diets¹

Fatty acid	Diets				
	SO	SU	SA	LI	BT
% total fatty acids					
14:0	0.2	0.2	3.3	0.2	2.2
16:0	10.6	7.7	13.1	6.9	20.7
16:1(n-7)	0.1	0.1	4.0	0.1	2.1
18:0	4.3	3.6	3.6	3.9	18.2
18:1(n-9)	21.0	29.4	20.7	18.0	30.7
18:2(n-6)	53.2	50.9	19.5	25.4	18.1
18:3(n-3)	7.1	3.8	5.9	42.9	2.6
20:1(n-9)	0.2	0.4	4.4	0.2	0.2
20:5(n-3)	0	0	6.1	0	0
22:5(n-3)	0	0	2.4	0	0
22:6(n-3)	0	0	7.5	0	0
Total PUFA	60.3	54.7	41.4	68.3	20.7
Total (n-3) PUFA	7.1	3.8	21.9	42.9	2.6
Total (n-6) PUFA	53.2	50.9	19.5	25.4	18.1
(n-6):(n-3) PUFA	7.5	13.4	0.9	0.6	7.0

¹ The CT had the same fatty acid composition as the SO diet. The CT diet contained 3% fat; the experimental diets contained 13% fat. Only the most abundant fatty acids are listed.

by centrifugation, resuspended in FACS-buffer (PBS, 1% BSA), and incubated with fluorescent-labeled antibodies for 1 h. The following monoclonal antibodies were used: Armenian hamster anti-mouse CD3-PerCPy5.5, rat anti-mouse CD4-FITC, rat anti-mouse CD8a-APC, rat anti-mouse CD69-PE, rat anti-mouse CD62L-PE, rat anti-mouse F4/80-FITC, rat anti-mouse CD11b-PerCPy5.5, rat anti-mouse CD94-FITC, rat anti-mouse NK1.1-APC, rat anti-mouse CD25-PE, and rat anti-mouse Forkhead box P3 (FoxP3)-APC. After incubation, the cells were washed 3 times and resuspended in 100 μ L FACS buffer. Cells that were used for analysis of CD3/CD4/CD25/FoxP3 expression were subsequently permeabilized using Fix/Perm buffer for 30 min at 4°C. Next, cells were washed twice with Perm/Wash buffer for 10 min. Cells were then resuspended in 100 μ L blocking buffer for 15 min. Subsequently, cells were stained with anti-FoxP3 for 30 min. Cells were washed twice and resuspended in FACS buffer. FACS analysis was carried out on a Becton Dickinson FACS Calibur flow cytometer and data were analyzed using FCS Express version 3 software (De Novo Software).

Statistical analysis. All data are expressed as means $\pm$ SEM. Statistical analyses were performed using SPSS 15.0 (SPSS Benelux). Differences were considered significant at $P < 0.05$. Variables were checked for Gaussian distribution with the Shapiro-Wilkes test. Levene's test for equality of variance was used to estimate the probability that treatment groups had different variances. One-way ANOVA was used to detect differences among multiple dietary groups at a time if all conditions were met, and Tukey's test was used to identify significant differences between the means. If not all conditions were met, the nonparametric Kruskal-Wallis test was performed, using Dunn's test to determine significant differences between the means. BALB/c and C57BL/6 mice fed the same diet were compared at 8 wk and 13 wk using an independent t -test when variances were homogeneous. When variances were heterogeneous, a nonparametric Mann-Whitney U test was performed. Paired t tests were used to identify changes in RBC fatty acids over time.

Results

ASR and DTH response. The total dietary fat concentration had no effect on the ASR (Fig. 2A) or DTH (Fig. 2B), the CT and SO groups did not differ; these 2 diets had the same fatty acid composition but differed in total fat concentration (CT, 3%; SO, 13%). In the OVA-sensitized mice, the ASR (i.e. the allergic immune response) was significantly attenuated in the SA group relative to all other groups, whereas in those vaccinated with influenza, the DTH was significantly greater in the SA group than in all other groups. In the LI groups, the ASR and DTH were not affected relative to the CT, SO, and SU groups,

although the LI diet had a similar (n-6):(n-3) PUFA ratio as the SA diet but contained a high level of ALA, the plant (n-3) PUFA, and no EPA or DHA. In the OVA-sensitized mice, the ASR was significantly greater in the BT group than in the other groups.

Fatty acid composition of RBC. Because the differences in allergic and vaccination responses could be caused by differences in plasma membrane fatty acid composition, the PUFA concentration of RBC was analyzed. In the SA and LI groups, the RBC PUFA concentration reflected the dietary fatty acid composition at 8 wk (age at the time of immunization) and 13 wk (age at end of the experiment) in both the OVA-sensitized mice (Table 2) and in those vaccinated with influenza (Table 3). In both experiments, the LI groups had higher ALA and EPA levels and lower AA levels compared to the other groups, except for the SA group at both 8 and 13 wk of age. Similarly, in both experiments, the SA groups had higher EPA and DHA levels and lower AA levels compared to the other groups, except for the LI group at both time points. The other diets had less marked effects on the RBC PUFA concentration.

While all OVA-sensitized BALB/c mice other than the SA group accumulated AA, and all accumulated DHA, over time, (Table 2), all influenza-vaccinated C57BL/6 mice had lower AA levels at the age of 13 wk compared to 8 wk (Table 3). DHA levels also decreased significantly in the C57BL/6 mice in the CT, SO, SU, and SA groups. C57BL/6 mice in the SA group also had lower EPA levels at 13 wk of age compared to the time of immunization at 8 wk of age (Table 3). This difference in PUFA accumulation resulted in higher levels of AA and DHA in C57BL/6 mice at 8 wk of age than in the BALB/c mice fed the same diet ($P \leq 0.01$). At the end of the experiment, however, the AA and DHA levels in the C57BL/6 mice were lower compared to the BALB/c mice fed the same diet ($P < 0.01$).

Immune cell subsets in lymphoid tissues. In thymus and spleen, mean percentages of CD3⁺CD4⁺ (T-helper) cells, CD3⁺CD4⁺CD69⁺ (activated T-helper) cells, CD3⁺CD4⁺CD62L⁺ (naïve T-helper) cells, CD3⁺CD8⁺ (cytotoxic T-cells), and CD3⁺CD4⁺CD25⁺FoxP3⁺ cells (T regulatory cells) did not differ among the groups in either experiment (data not shown). Similarly, the percentages of CD19⁺ B-cells and CD94⁺ NK cells and F4/80⁺ macrophages in the spleen and bone marrow were not affected by the dietary treatments (data not shown).

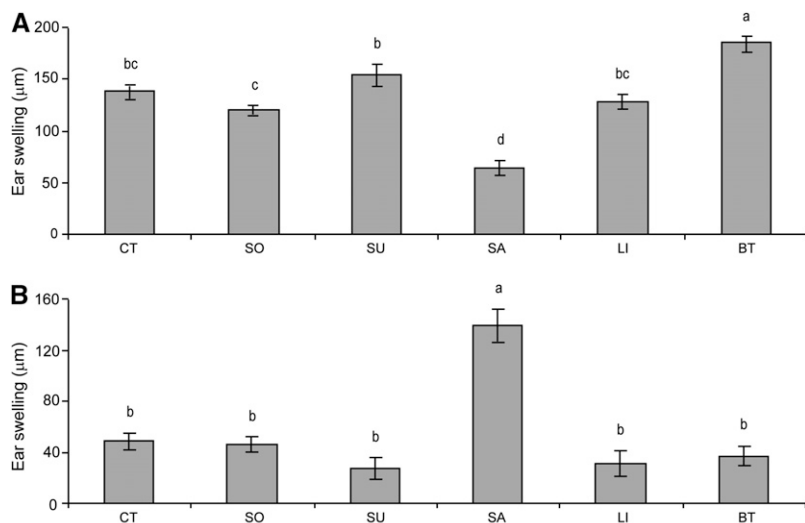


FIGURE 2 T helper-2-mediated ASR in OVA-sensitized male BALB/c mice (A) and T helper-1-mediated DTH responses in influenza-vaccinated male C57BL/6 mice (B) that consumed diets differing in fatty acid composition for 5 and 6 wk, respectively. Values are means $\pm$ SEM, $n = 6$. Means without a common letter differ, $P < 0.05$.

TABLE 2 RBC fatty acid composition in OVA-sensitized male BALB/c mice fed diets differing in fatty acid composition at the time of immunization at 8 wk and at the end of the experiment at 13 wk of age¹

Diet	LA		ALA		AA		EPA		DHA	
	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk
% total fatty acids										
CT	6.8 ± 0.4 ^c	10.7 ± 0.2 ^{b*}	0.2 ± 0.0 ^c	0.2 ± 0.0 ^c	6.2 ± 0.2 ^a	12.7 ± 0.2 ^{a*}	0.2 ± 0.1 ^c	0.2 ± 0.0 ^b	1.9 ± 0.1 ^b	4.9 ± 0.1 ^{c*}
SO	10.2 ± 0.7 ^a	14.2 ± 0.3 ^{a*}	0.3 ± 0.0 ^b	0.3 ± 0.0 ^b	5.7 ± 0.3 ^a	11.8 ± 0.2 ^{a,b*}	0.0 ± 0.0 ^c	0.2 ± 0.0 ^{b*}	2.1 ± 0.1 ^b	5.5 ± 0.1 ^{b*}
SU	8.9 ± 0.6 ^b	13.3 ± 0.4 ^{a*}	0.1 ± 0.0 ^c	0.2 ± 0.0 ^c	6.1 ± 0.5 ^a	12.4 ± 0.4 ^{a,b*}	0.0 ± 0.0 ^c	0.1 ± 0.0 ^{b*}	1.8 ± 0.2 ^b	4.5 ± 0.1 ^{c*}
SA	7.0 ± 0.0 ^c	9.5 ± 0.4 ^{b*}	0.2 ± 0.0 ^{b,c}	0.3 ± 0.1 ^{bc}	3.3 ± 0.1 ^b	4.0 ± 0.1 ^d	1.8 ± 0.1 ^a	3.7 ± 0.2 ^{a*}	4.0 ± 0.2 ^a	10.2 ± 0.3 ^{a*}
LI	8.9 ± 0.5 ^b	13.6 ± 0.3 ^{a*}	1.7 ± 0.3 ^a	1.6 ± 0.1 ^a	3.5 ± 0.1 ^b	5.4 ± 0.1 ^{c*}	0.7 ± 0.1 ^b	2.2 ± 0.1 ^{a*}	2.0 ± 0.1 ^b	6.5 ± 0.3 ^{b*}
BT	7.5 ± 0.6 ^{b,c}	10.5 ± 0.7 ^{b*}	0.2 ± 0.1 ^{b,c}	0.2 ± 0.0 ^{b,c}	6.1 ± 0.3 ^{a,b}	11.6 ± 0.2 ^{b*}	0.3 ± 0.1 ^c	0.5 ± 0.1 ^{b*}	2.0 ± 0.1 ^b	5.0 ± 0.3 ^{b,c*}

¹ Data are represented as means ± SEM, *n* = 6. Means at a time without a common letter differ, *P* < 0.05. *Different from 8 wk, *P* < 0.05.

Ex vivo cytokine production and plasma antibody concentrations. Cytokine secretion after splenocyte restimulation did not differ significantly among the dietary groups in either experiment (data not shown). Although detectable levels of OVA-specific IgE, IgG2a, and IgG1 and influvac-specific IgG2a and IgG1 were measured in the plasma of OVA-allergic mice and influenza-vaccinated mice, respectively, the groups did not differ in either experiment (data not shown).

Discussion

The present study indicates that the quality of dietary fat can influence the immune response. Using mouse models for allergy and vaccination responses, we showed that a SA diet has a beneficial effect on both the T-helper 2-mediated allergic response and on the T-helper 1-mediated vaccination response. In contrast to the SA diet, the LI diet did not affect the allergic or vaccination response, even though this diet had a (n-6):(n-3) PUFA ratio similar to that of the SA diet and an even higher total PUFA concentration as compared to the SA diet. The LI diet, however, was high in ALA but deficient in EPA and DHA, in contrast to the SA diet. The difference in effectiveness between these 2 high-(n-3) PUFA diets might be caused by the differences in elevation of the plasma membrane EPA and DHA concentrations by these diets. The LI group had significantly lower EPA and DHA levels than the SA group at the time of immunization and lower DHA levels at the time of ASR/DTH measurement. These differences in plasma membrane PUFA may have led to differences in eicosanoid production. Eicosanoids play a key role in inflammatory responses (10) and their production varies due to different availability of their precursors, which in turn is likely to affect the immune response to OVA/influvac.

In the OVA-sensitized BALB/c mice, the LI diet increased RBC EPA and DHA levels over time, as the LI group had higher levels of these lcPUFA at the end of the experiment at 13 wk of age compared to the levels at sensitization at 8 wk of age. At the end of the experiment, the EPA and DHA levels in these mice were comparable to the levels in the SA group at the time of sensitization. This suggests that the ASR was influenced by EPA and DHA levels at the time of sensitization and thus feeding the LI diet for a longer period of time before sensitizing the mice to OVA might also reduce the allergic response, as observed in the SA group. Whereas the LI and SA diets altered the RBC membrane composition, AA levels did not increase and EPA and DHA levels did not decrease in the RBC of the SU group consuming the high-LA SU diet, which might underlie the fact that both the allergic as well as the vaccination response in these mice did not differ from the CT group. AA is the precursor of proallergic PGE₂ (5), so an increase in membrane AA might increase allergy severity. However, high dietary LA did not increase AA levels. The BT group had a significantly greater allergic response compared to all other dietary groups. This enhanced allergic response was probably not due to differences in eicosanoid precursors, because the RBC fatty acid concentration of the mice consuming the BT diet was similar to the control mice. Dietary SFA, however, can activate the immune response directly via stimulation of Toll-like receptor signaling and thereby augment an inflammatory reaction (24). The ASR and the DTH did not differ between the CT and SO groups. The CT and SO diets had the same fatty acid composition, but the SO diet contained 13% fat and the CT diet 3% fat, indicating that the quality, and not the quantity, of the dietary fat is of importance in influencing the immune response. It should be noted that whereas OVA-sensitized BALB/c mice, except those in the

TABLE 3 RBC fatty acid composition in influenza-vaccinated C57BL/6 mice fed diets differing in fatty acid composition at the time of immunization at 8 wk and at the end of the experiment at 13 wk of age¹

Diet	LA		ALA		AA		EPA		DHA	
	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk	8 wk	13 wk
% total fatty acids										
CT	9.3 ± 0.2 ^c	10.5 ± 0.3 ^{c*}	0.2 ± 0.0 ^c	0.2 ± 0.0 ^b	8.5 ± 0.3 ^a	6.7 ± 0.6 ^{a*}	0.1 ± 0.0 ^c	0.1 ± 0.0 ^{c,d}	2.8 ± 0.1 ^b	2.1 ± 0.2 ^{b*}
SO	14.9 ± 0.4 ^a	15.3 ± 0.8 ^a	0.5 ± 0.1 ^b	0.3 ± 0.0 ^{b*}	9.1 ± 0.6 ^a	6.2 ± 0.6 ^{a*}	0.2 ± 0.0 ^c	0.1 ± 0.0 ^{c,d}	3.6 ± 0.4 ^b	2.4 ± 0.3 ^{b*}
SU	12.7 ± 0.2 ^b	12.6 ± 0.8 ^b	0.2 ± 0.0 ^c	0.2 ± 0.0 ^{c*}	10.9 ± 0.9 ^a	7.0 ± 0.9 ^{a*}	0.0 ± 0.0 ^d	0.0 ± 0.0 ^d	3.6 ± 0.4 ^b	1.9 ± 0.2 ^{b*}
SA	9.1 ± 0.2 ^c	8.8 ± 0.5 ^c	0.4 ± 0.0 ^{b,c}	0.2 ± 0.0 ^{b*}	4.5 ± 0.2 ^b	2.1 ± 0.2 ^{b*}	3.8 ± 0.2 ^a	2.4 ± 0.3 ^{a*}	7.1 ± 0.5 ^a	4.1 ± 0.5 ^{a*}
LI	12.8 ± 0.3 ^b	14.7 ± 0.8 ^a	2.4 ± 0.2 ^a	1.6 ± 0.1 ^{a*}	4.9 ± 0.5 ^b	2.8 ± 0.3 ^{b*}	1.9 ± 0.2 ^b	1.7 ± 0.2 ^b	3.5 ± 0.4 ^b	2.6 ± 0.4 ^b
BT	9.6 ± 0.3 ^c	11.4 ± 0.4 ^{ab}	0.2 ± 0.0 ^c	0.2 ± 0.0 ^{b,c}	9.5 ± 0.6 ^a	7.6 ± 0.9 ^{a*}	0.2 ± 0.0 ^c	0.2 ± 0.0 ^c	3.4 ± 0.3 ^b	2.8 ± 0.4 ^b

¹ Data are represented as means ± SEM, *n* = 6. Means at a time without a common letter differ, *P* < 0.05. *Different from 8 wk, *P* < 0.05.

SA group, accumulated AA, EPA, and DHA over time, most flu-vaccinated C57BL/6 mice had lower AA and DHA levels at the end of the experiment compared to the time of immunization. This marked difference in PUFA accumulation was also detected in another experiment using these 2 mouse strains (23), indicating that the strains handle PUFA differently.

In the current study, the diets did not affect cell populations in the spleen and thymus, but it is possible that at the time of sensitization/vaccination differences did occur, which could ultimately lead to differences in the ASR and the DTH, respectively. Another explanation may lie in the difference between local and systemic effects. The fact that no differences in cytokine production by restimulated splenocytes were observed does not mean that no differences in local cytokine production at the site of antigen exposure (i.e. the ear) occurred. Similarly, although IgE levels were similar among the groups, it is possible that during the ASR, differences in local production of inflammatory mediators by mast cells as a response to OVA exposure led to the lower ASR in the SA group. This is in line with results obtained in other studies where dietary intervention also led to a reduction in in vivo allergic symptoms, but other immune variables such as IgE levels and cytokine expression levels were not significantly different (23,25,26). Future studies are necessary to further elucidate the underlying mechanism(s) involved in the effects on ASR and DTH observed here. In human studies, many immunomodulatory effects of lPUFA have also been found, and some intervention trials show that supplementation with lPUFA may have a preventive effect on the development of allergies and the occurrence of infections [reviewed by (27)]. Although the Western human diet contains less EPA and DHA compared to a mouse diet supplemented with 10% salmon oil, it is very feasible that a diet enriched in (n-3) lPUFA may contribute to a healthy immune response. In conclusion, the findings obtained in this study show that a SA-based diet can improve the T-helper 1-mediated vaccination response and decrease the T-helper 2-mediated allergic response.

Acknowledgments

J.G. and P.C.C. designed the research; A.H., N.V., A.L.F., B.C.E., and G.A.H. conducted the research; A.H., N.V., and A.L.F. analyzed the data and performed statistical analysis; A.H., N.V., and P.C.C. wrote the paper; and P.C.C. and J.G. had primary responsibility for final content. All authors read and approved the final manuscript.

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