

RESEARCH ARTICLE

Effects of supplementing different ratios of omega-3 and omega-6 fatty acids in western-style diets on cow's milk protein allergy in a mouse model

Cin L. Thang¹, Joyce I. Boye², Hai Ning Shi³ and Xin Zhao¹

¹Department of Animal Science, Macdonald Campus, McGill University, Lakeshore, Quebec, Canada

²Food Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, Quebec, Canada

³Mucosal Immunology Laboratory, Massachusetts General Hospital, Charlestown, MA, USA

Scope: This study investigated the effects of supplementing different ratios of omega-6 and omega-3 fatty acids (O6H = 10:1, O3O6 = 4:1, and O3H = 1:4) to western-style diets on cow β -lactoglobulin (BLG) induced allergic reactions in Balb/c mice.

Methods and results: Three-week-old mice were randomly assigned to three diet groups ($n = 20$ /group). At 9 wk of age, half of the mice from each dietary treatment ($n = 10$) were intraperitoneally (i.p.) sensitized with three weekly doses of BLG and alum while the remaining half from each group was sham sensitized (controls). One week after the final sensitization, all mice were orally challenged with BLG. Elevated BLG-specific serum IgS were observed in all sensitized and challenged mice. IFN- γ , MCP-1, and IL-12p40 concentrations from lymphocytes of mesenteric lymph nodes were highest in O3H mice, compared to O3O6 and O6H mice. O6H mice had the highest IL-4 concentrations from splenic lymphocytes and a significantly lower rectal temperature after the challenge in comparison to O3O6 and O3H mice.

Conclusions: Our results suggest that the ω -3 PUFA rich diets alleviated the severity of allergic reactions, and may modulate immune response toward T helper cell (Th)1-favoured immune response while the ω -6 PUFA rich diet exhibited no allergy alleviation with a stronger Th2 polarized immune response.

Keywords:

β -Lactoglobulin / Food allergy / Mouse model / Omega 3 fatty acids / Western-style diet

Received: January 21, 2013

Revised: March 30, 2013

Accepted: April 21, 2013

1 Introduction

Food allergy is an immune-mediated hypersensitive response in susceptible individuals after ingestion of food that is considered harmless to others. Prevalence of food allergy and

its outgrowing age, particularly in the children of developed nations, are significantly increased in the past three decades. Food allergy-associated menaces, starting from discomfort lifestyle to life-threatening anaphylaxis reactions as well as its growing incidence rate and current incurable status, pose an alarming risk to current patients and future generations; and cause a huge burden to families and social communities. Food allergy patients have also increased susceptibility to aeroallergens [1] and asthma is diagnosed in over 30% of children with food allergy [2]. Today, cow's milk protein allergy (CMA) still remains as the major food allergy to children and youth of developed countries. Among many possible reasons, early introduction of cow's milk protein to infants either as replacement or reinforcement for breast feeding coupled with consumption of recently widespread western-style diets may partake a substantial role in steady increase of CMA. The western-style diet, also known as the standard American diet, is generally defined as a diet that contains a high level of energy (derived mainly from highly processed and refined

Correspondence: Professor Xin Zhao, Faculty of Agriculture and Environmental Sciences, Department of Animal Science, Macdonald Campus of McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, H9X 3V9, QC, Canada

E-mail: xin.zhao@mcgill.ca

Fax: +1-514-398-7964

Abbreviations: AA, arachidonic acid; BLG, β -lactoglobulin; CMA, cow's milk protein allergy; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; i.p., intraperitoneal injection; LCPUFA, long-chain polyunsaturated fatty acids; MLNs, mesenteric lymph nodes; O6H, ω -6 high diet (ω -6/ ω -3; ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3; ratio 4:1); O3H, ω -3 high diet (ω -6/ ω -3; ratio 1:4); OVA, ovalbumin; sIgA, secretory IgA; Th, T helper cell

grains, sugar, and red meat), a high level of fat (usually high in saturated and trans-fatty acids but low in MUFAs and PUFAs), and a high level of sodium [3]. In regard to increased incidence of allergy, the composition of fatty acids from the western-style diet, rather than its energy and sodium contents, is more concerned.

Variations of long-chain polyunsaturated fatty acid (LCP-UFA) composition, particularly the rapid increase of ω -6/ ω -3 ratio in diets, have been considered as one of the major reasons for increased allergy incidence. Eaton et al. estimated that the ω -6/ ω -3 ratio in the diet of human beings in the Paleolithic period was 0.79 and remained relatively the same for millions of years [4]. However, the ω -6/ ω -3 ratio suddenly sky rocketed within the last century due to rapid dietary changes. Currently the ω -6/ ω -3 ratio in western societies has been estimated as (15–17):1 [5], while the suggested optimal dietary ratio of ω -6/ ω -3 is 4:1 [6]. The unbalanced ω -6/ ω -3 ratio in the diet is due to increased consumption of ω -6-rich foods, e.g., linoleic acid (C18:2 ω -6)-rich vegetable oils and decreased consumption of ω -3-rich foods, such as fish and green vegetables, flaxseed, rapeseed oil, or nuts that have a relatively high content of α -linolenic acid (ALA, C18:3 ω -3). This substantial shifting of dietary ω -6/ ω -3 ratios coincides with the recent higher food allergy incidence.

The association between a higher ω -6/ ω -3 ratio and allergy has not been fully explained. LCPUFAs serve as substrates for inflammatory mediators. It has been suggested that diets rich in ω -6 LCPUFAs generate a high level of potent inflammatory mediators, such as prostaglandin E2 and leukotriene 4, and lead to a T helper cell (Th)2-favored immune response. On the other hand, increased intake of ω -3 has been reported to reduce inflammatory reactions by producing less potent inflammatory mediators, such as prostaglandin E3 and leukotriene 5. Moreover, ω -3-derived resolvins, particularly eicosapentaenoic acid (EPA)-derived resolvins, is known to induce an anti-inflammatory effect by binding to Chemr23 receptor on APCs and subsequently attenuating NF κ B activation [7] and reduce allergic airway responses in mice [8]. The epidemiological finding of the linkage between reduced ω -3 LCPUFA intake and increased prevalence of allergic diseases and the discovery of anti-inflammatory effects contributed by ω -3 LCPUFA-derived molecules have generated enormous attention to study the role of these fatty acids in allergic diseases [9]. The results obtained so far are inconsistent [10–14]. To our knowledge, the effects of dietary fatty acids on allergy have been studied selectively in a mouse model of allergic airway disease using ovalbumin (OVA). There is no report about effects of dietary fatty acids on cow's milk protein induced food allergy.

The dietary content of ω -3 and ω -6 LCPUFAs in the diet can be adjusted in two ways: by adding essential fatty acids, linoleic acid and ALA (precursors of LCPUFAs), or by adding fish oils that contain preformed EPA and docosahexaenoic acids (DHA). Although mammals can synthesize EPA and DHA through its precursor ALA, its bioconversion rate, through desaturation and elongation processes, is limited

since arachidonic acid (AA) is also metabolized by the same pathway [15]. A recent review indicated that a long-term intake of ALA has increased the proportions of EPA, but not DHA, in plasma or cell lipids [15]. Therefore, we have used fish oils, rather than ALA-containing oils, as the source of ω -3 LCPUFAs in our experimental diets.

Studies about effects of maternal supplementation with ω -3 during pregnancy on offspring have gained attention due to the findings of its possible preventive effect on asthma and allergy from randomized, controlled trials [13, 16], while therapeutic approach to asthma with ω -3 (fish oil) was unsuccessful [14]. However, preventive potentials of ω -3 supplementation directly to new born animals, not through mothers, are less studied. We hypothesize that feeding young mice with western-style diets that contain a high level of ω -3 LCPUFAs (EPA and DHA) may have a direct effect on Th switching and T-cell-mediated immune responses, which may subsequently reduce allergic reactions to milk proteins. The purpose of this mouse model study was to evaluate whether increasing ω -3 PUFAs in western-style diets can reduce β -lactoglobulin (BLG)-induced food allergy.

2 Materials and methods

2.1 Animals and experimental design

Three-week-old female Balb/C mice (Charles River Breeding laboratories, St. Constant, Quebec, Canada) were housed in individual cages under a 12L:12D lighting cycle, 20–24°C range of ambient temperature, and 40–70% of relative humidity. Mice were randomly assigned to three groups of 20 mice each that receive a ω -6 high diet (O6H), a ω -6 and ω -3 balanced diet (O3O6), and a ω -3 high diet (O3H). Mice were given ad libitum access to feed and water. At 9 wk of age, half of the mice from each dietary treatment ($n = 10$) were intraperitoneally (i.p.) sensitized with three weekly doses of 50 μ g BLG and 2 mg alum in PBS (Fig. 1). The remaining half from each group was sham sensitized with alum in PBS and used as controls. One week after the final sensitization, all mice were orally challenged with 60 mg of BLG per mouse. Animal care use and protocols were approved by the McGill University Animal Care Committee (protocol # 5940).

2.2 Experimental diets

Energy- and fat-rich western-style rodent diets with different ratios of ω -6/ ω -3 (10:1 (O6H), 4:1 (O3O6), and 1:4 (O3H)) were formulated (Research diets Inc., New Brunswick, NJ) by slight modification of a previously described western-style rodent formula (40% kcal fat/15% protein/45% carbohydrate; 4.62 kcal/g) [17, 18]. Energy contribution of each experimental diet was 45% from carbohydrate, 20% from protein, and 35% from fat (Table 1). The total energy level of each diet was 4.24 kcal/g. Our diets are different from a standard laboratory rodent diet, which usually contains 3.0 kcal/g of energy

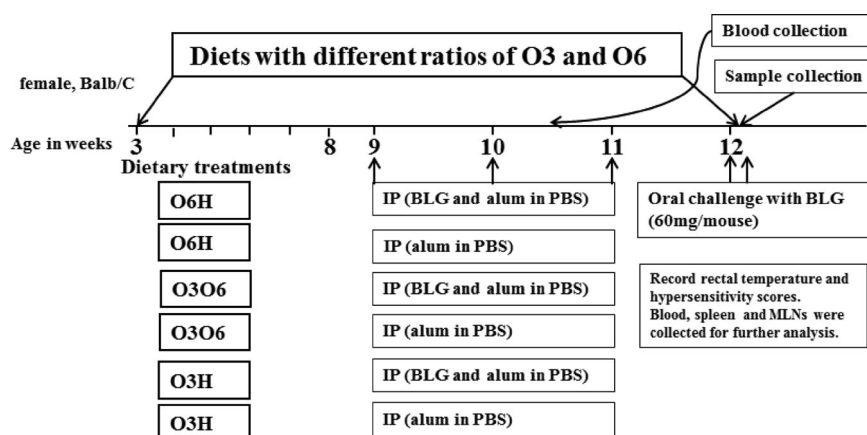


Figure 1. Experimental design. Three-week-old mice were divided into six groups ($n = 10$ per group) and fed with high-energy diets containing the following three different ratios of ω -6 and ω -3 throughout the experiment. O6H: ω -6 high diet (ω -6/ ω -3, ratio 10:1); O3O6: ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1), and O3H: ω -3 high diet (ω -6/ ω -3, ratio 1:4). After three doses of i.p. sensitization with β -lactoglobulin (BLG) and alum, food allergy was induced by oral administrations of 60 mg BLG. Control groups for each diet were sham sensitized with alum in PBS. Three days after second i.p. sensitization, tail blood samples were collected. Hypersensitivity scores and rectal temperatures were recorded within 1 h after final oral challenge. Three hours after final oral challenge, mice were sacrificed by CO_2 inhalation and blood, tissues, and organ samples were collected for further analyses.

Table 1. Calculated fatty acids composition (grams per 100 g of total fatty acids)

Fatty acids	O6H	O3O6	O3H
C14	1.33	2.66	6.58
C16	8.04	9.68	14.5
C16:1	1.90	3.73	9.37
C16:2	0.32	0.63	1.58
C16:3	0.32	0.57	1.46
C16:4	0.32	0.57	1.46
C18	2.34	2.41	2.53
C18:1 (ω 9)	11.52	11.08	9.68
C18:2 (ω 6), LA	63.61	48.86	5.06
C18:3 (ω 3), ALA	0.38	0.63	1.46
C18:4 (ω 3)	0.57	1.20	2.97
C20:2 (ω 6)	0.06	0.06	0.19
C20:3 (ω 6)	0.06	0.13	0.38
C20:4 (ω 6)	0.38	0.82	2.03
C20:5 (ω 3), EPA	2.72	5.44	13.6
C21:5 (ω 3)	0.13	0.32	0.76
C22:4 (ω 6)	0.06	0.06	0.25
C22:5 (ω 3)	0.57	1.08	2.72
C22:6 (ω 3), DHA	1.96	3.99	9.81
SFA (g)	12.09	15.51	25.6
MUFA (g)	13.86	15.70	21.1
PUFA (g)	71.46	64.43	43.5
ω -3: ω -6	10	4	0.25

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SFA, saturated fatty acids; O6H, ω -6 high diet (ω -6/ ω -3, ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1), and O3H, ω -3 high diet (ω -6/ ω -3, ratio 1:4).

derived from protein, fat, and carbohydrate with the percentage of 32, 14, and 54, respectively (Teklad Rodent Diet-8604, Harlan Laboratories).

Menhaden oil was used for the source of ω -3 PUFAs and safflower oil was used for the source of ω -6 PUFAs. The diet manufacturer has added antioxidant *tert*-butylhydroquinone

(tBHQ) to the oils to prevent deterioration of fatty acids [19]. Upon arrival, the diets were packed into daily portions in zip-lock bags, sealed and stored at -80°C . Mice were fed daily with the fresh pellets throughout the experimental period. Feed intake and body weight from all treatment groups were recorded.

2.3 Reagents

Horseradish peroxidase conjugated anti-mouse antibodies were obtained from Bethyl Laboratories (Montgomery, TX, USA). Purified anti-mouse Igs were obtained from BD Pharmingen (San Diego, CA, USA) and ABTS peroxidase substrate was purchased from Mandel Scientific (Guelph, Ontario, Canada). Bio-Plex Pro Mouse Cytokine Assay kits (Bio-Rad, Hercules, CA, USA) were used to detect cytokines. All other chemicals were purchased from either Sigma (St. Louis, MO, USA) or Fisher Scientific (Nepean, ON, Canada).

2.4 Recording rectal temperature and hypersensitivity scores

Rectal temperatures were recorded at three time points—just before final oral challenge (0 min), 30 and 45 min postoral challenge—using BAT-12 Microprobe Thermometer with a mouse-specific rectal probe (Physitemp Instruments, Inc., Clifton, NJ, USA). Within an hour following the final oral BLG challenge, hypersensitivity symptoms were scored by using the score system as described by Schouten et al. [20]. The scores were as follows: 0 = no symptom; 1 = scratching and rubbing around the nose and head; 2 = reduced activity; 3 = activity after prodding and puffiness around the eyes and mouth; 4 = no activity after prodding, labored respiration, and cyanosis around the mouth and the tail; and 5 = death.

2.5 Fecal and serum samples collection

Fresh fecal pellets were collected at day 51 of age by transferring mice into new cages that were lined with moist paper towels. Fecal pellets were immediately stored in -80°C until further analysis. Three days after the second i.p. sensitization, blood samples were collected from tail vein. Three hours after the final oral BLG challenge, mice were euthanized by CO_2 inhalation and blood samples were collected by intracardiac puncture. All blood samples were collected into serum separator tubes (Sarstedt, Montreal, Quebec, Canada). The serum portion was separated by centrifugation at $10\,000 \times g$ for 5 min at 20°C . Serum samples were then aliquoted into Eppendorf tubes and stored at -20°C until analysis.

2.6 Determination of serum BLG-specific Igs, total IgE, and fecal IgA

BLG-specific serum IgG1, IgG2a, and IgA as well as total IgE and total fecal IgA were detected by ELISA as previously described [21]. Serum samples were diluted 1:100 for BLG-specific serum IgE, 1:200 for total IgE, and 1:1000 for others before being used in the assays. All analyses were performed in duplicates or triplicates and the mean values were used. Relative Ig concentrations were determined by extrapolation from standard curves with purified mouse Ig standards. For detection of BLG-specific serum IgE, the SignalBoost Immunoreaction Enhancer Kit was used to dilute serum and HRP-conjugated anti-mouse IgE.

Fecal supernatants were prepared from fecal samples as described by Adel-Patient et al. [22]. Fecal pellets (100 mg) were placed in 1 mL of PBS containing 0.01% v/v of the protease inhibitor cocktail (Sigma-Aldrich). The mixture was vortexed, centrifuged at $16\,000 \times g$ for 10 min and the supernatants were collected. Undiluted supernatants were used to determine fecal secretory IgA by ELISA.

2.7 Lymphocyte isolation and in vitro restimulation with anti-CD3

Three hours after the final oral BLG challenge, mice were sacrificed and lymphocytes from spleen and mesenteric lymph nodes (MLNs) were collected as previously described [23]. Briefly, spleens and MLNs were aseptically removed. Lymphocyte suspensions were obtained by pressing the cells through a $70\text{ }\mu\text{M}$ nylon cell strainer (Falcon; BD Labware) in complete DMEM. After lysing red blood cells, the cells were washed and cultured (5×10^6 cells/mL) in 24-well plates in the presence or absence of anti-CD3 monoclonal antibody (MAb; $10\text{ }\mu\text{g/mL}$) and BLG ($100\text{ }\mu\text{g/mL}$) for 72 h at 37°C . Culture supernatants were collected, labeled, and stored at -20°C until cytokines analysis.

2.8 Cytokine measurements from lymphocytes culture supernatants

Cytokines from lymphocytes culture supernatants were analyzed by Mouse Cytokine 23-plex Assay kits. After following the manufacturer's protocol, beads were read with a Bio-Plex Reader and data were analyzed with the Bio-Plex Manager 2.0 software (Bio-Rad Laboratories).

2.9 Serum fatty acid analysis

Serum fatty acid composition was analyzed by GC following a recently developed protocol with minor modifications [24]. This protocol is particularly useful for our experiment since it requires only 100 μL of serum. Briefly, 100 μL each of serum and an internal standard, and 1.5 mL of methanolic HCl (3 N), containing 75 $\mu\text{g/mL}$ of butylated hydroxytoluene, were mixed in the closed glass vial. Heptadecanoic acid, C 17:0, (Nu-Check Prep Inc., Elysian, MN, USA) was used as the internal standard. The vials were then shaken for 30 s and heated at 85°C for 45 min. When the vials were cooled down to room temperature, 0.5 mL of hexane was added to each vial. The vials were shaken for 30 s and upper hexane phase containing FAME was collected in 2 mL vials for GC analysis. Fatty acid composition of the FAME was measured by a CP-3900 GC (Varian, Palo Alto, CA, USA) using a capillary column (CP-7489, 100 m $\times$ 0.25 mm, 0.2 μm film) with a flame ionization detector and a splitless injection. Serum fatty acids were identified by comparing the retention times of their peaks with the fatty acid methyl mix standard (Supelco 37 Comp. FAME Mix 10 mg/mL in CH_2Cl_2 ; Supelco, Bellefonte, PA, USA).

2.10 Statistical analyses

Data were analyzed by using the PROC MIXED of SAS 9.3 (SAS Institute, 2003) except for the hypersensitivity scores data, which were analyzed using the Kruskal–Wallis test and the SigmaStat software (SPSS Inc. Chicago, IL, USA). Differences among treatment means were tested using the Scheffé multiple comparison test. Data are presented as means $\pm$ SEM. A *p*-value less than 0.05 was considered significantly different.

3 Results

3.1 Serum fatty acid compositions

To confirm the efficiency of fatty acid supplementation in the diets, serum fatty acid compositions were analyzed (Table 2). Comparison of serum ω -3 and ω -6 fatty acids between control and BLG-sensitized mice that received the same diet generally show similar levels of fatty acids, with the exception of C20:5

Table 2. Serum fatty acid composition in mice fed high-energy diets containing different ratios of ω 6 and ω 3 fatty acids

Fatty acid	O6H (c)	O6H	O3O6 (c)	O3O6	O3H (c)	O3H
C14:0	1.01 $\pm$ 0.07 ^{c)}	0.93 $\pm$ 0.07 ^{c)}	1.26 $\pm$ 0.07 ^{b,c)}	1.22 $\pm$ 0.05 ^{b,c)}	1.63 $\pm$ 0.08 ^{a,b)}	1.79 $\pm$ 0.05 ^{a)}
C16:0	21.32 $\pm$ 0.37 ^{c)}	21.44 $\pm$ 0.37 ^{c)}	25.07 $\pm$ 0.37 ^{b)}	25.86 $\pm$ 0.30 ^{b)}	29.88 $\pm$ 0.43 ^{a)}	31.22 $\pm$ 0.30 ^{a)}
C16:1	0.91 $\pm$ 0.08 ^{c,d)}	0.88 $\pm$ 0.08 ^{d)}	1.32 $\pm$ 0.08 ^{c)}	1.07 $\pm$ 0.07 ^{c,d)}	3.79 $\pm$ 0.10 ^{a)}	3.31 $\pm$ 0.07 ^{b)}
C18:0	12.76 $\pm$ 0.37	13.62 $\pm$ 0.37	13.47 $\pm$ 0.37	14.38 $\pm$ 0.31	12.89 $\pm$ 0.43	13.89 $\pm$ 0.31
C18:1 ω 9	6.21 $\pm$ 0.38 ^{a,b)}	5.91 $\pm$ 0.38 ^{b)}	6.71 $\pm$ 0.38 ^{a,b)}	5.93 $\pm$ 0.31 ^{b)}	8.27 $\pm$ 0.44 ^{a)}	8.12 $\pm$ 0.31 ^{a)}
C18:2 ω 6 LA	40.62 $\pm$ 0.65 ^{a)}	40.12 $\pm$ 0.65 ^{a)}	31.94 $\pm$ 0.65 ^{b)}	29.21 $\pm$ 0.53 ^{b)}	12.15 $\pm$ 0.75 ^{c)}	11.21 $\pm$ 0.53 ^{c)}
C20:3 ω 6	0.79 $\pm$ 0.10 ^{b)}	0.72 $\pm$ 0.10 ^{b)}	1.33 $\pm$ 0.10 ^{a)}	1.39 $\pm$ 0.08 ^{a)}	0.74 $\pm$ 0.12 ^{b)}	0.36 $\pm$ 0.08 ^{b)}
C20:4 ω 6 AA	10.54 $\pm$ 0.46	10.35 $\pm$ 0.46	9.91 $\pm$ 0.46	11.36 $\pm$ 0.37	10.05 $\pm$ 0.53	9.76 $\pm$ 0.37
C20:5 ω 3 EPA	1.61 $\pm$ 0.16 ^{c)}	1.73 $\pm$ 0.16 ^{c)}	1.88 $\pm$ 0.16 ^{c)}	1.81 $\pm$ 0.13 ^{c)}	8.34 $\pm$ 0.19 ^{a)}	7.31 $\pm$ 0.13 ^{b)}
C22:6 ω 3 DHA	4.25 $\pm$ 0.21 ^{c)}	4.29 $\pm$ 0.21 ^{c)}	7.09 $\pm$ 0.21 ^{b)}	7.77 $\pm$ 0.17 ^{b)}	12.27 $\pm$ 0.25 ^{a)}	13.04 $\pm$ 0.17 ^{a)}
Total ω 6	51.95 $\pm$ 0.43 ^{a)}	51.19 $\pm$ 0.43 ^{a)}	43.18 $\pm$ 0.43 ^{b)}	41.96 $\pm$ 0.35 ^{b)}	22.93 $\pm$ 0.49 ^{c)}	21.32 $\pm$ 0.35 ^{c)}
Total ω 3	5.85 $\pm$ 0.25 ^{c)}	6.02 $\pm$ 0.25 ^{c)}	8.97 $\pm$ 0.25 ^{b)}	9.57 $\pm$ 0.21 ^{b)}	20.61 $\pm$ 0.29 ^{a)}	20.35 $\pm$ 0.21 ^{a)}
ω 6/ ω 3	8.88	8.51	4.81	4.38	1.11	1.05

Values are least squares means $\pm$ SEM. Fatty acid content is expressed as percentage of total fatty acids. The diet with "(c)" represents the control group without β -lactoglobulin (BLG) sensitization for that diet.

a,b,c) Values with different superscripts within the same row are different ($p \leq 0.05$).

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; O6H, ω -6 high diet (ω -6 ω -3, ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1); O3H, ω -3 high diet (ω -6/ ω -3, ratio 1:4).

ω -3 EPA levels in O3H diet supplemented mice. In O3H mice, the C20:5 ω -3 EPA levels in BLG-sensitized mice were lower than that in corresponding control mice. The serum ω -3 and ω -6 ratios reflected the ω -3 and ω -6 ratios in three diets.

3.2 Rectal temperature, hypersensitivity scores, body weights, and feed intake

A sudden decline of body temperature, itchy skin, and reduced activity are common signs of anaphylactic reactions. Body temperature recorded at 30 and 45 min after oral BLG challenge was compared with the initial body temperature. BLG-sensitized O6H mice had a significantly lower rectal temperature, while other groups had relatively similar temperatures in comparison to control mice (Fig. 2). After the final oral BLG challenge, all mice sensitized and challenged with BLG had slightly reduced activity with scratching behavior while control mice showed no visible symptoms (Fig. 3). There were no differences of hypersensitivity scores among all treatment groups.

The digestive system can also be affected by anaphylactic reactions. Body weight and feed intake were recorded for a period of 4 wk starting from 7 wk of age. Although no differences in body weight were observed, mice on O3H diets had a greater feed intake during week 7 and week 9 (Table 3).

3.3 Serum Igs

In comparison with control mice, sera in all BLG sensitized mice had significantly higher BLG-specific serum IgE, IgG1, and IgG, which are systemic markers for allergic reaction (Table 4, Figs. 4A, B, D). At the same time, mice receiving O3O6 and O3H diets had significantly higher concentrations

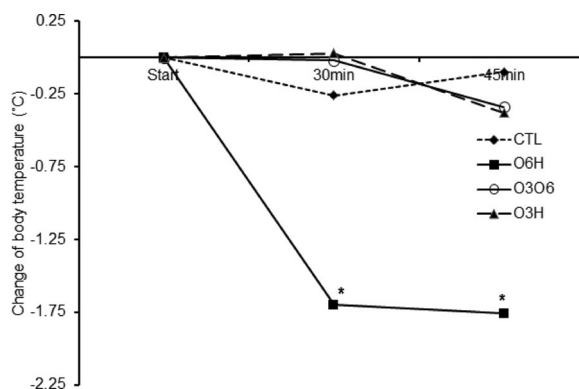


Figure 2. Changes of body temperature in mice after challenge with β -lactoglobulin (BLG). Rectal temperatures were recorded just before final oral BLG challenge (0 min), 35 min and 45 min postchallenge. The asterisk mark (*) designates statistically significant differences ($p \leq 0.05$) in comparison to control mice. CTL, control mice that received sham sensitization with alum in PBS; O6H, ω -6 high diet (ω -6/ ω -3, ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1); O3H, ω -3 high diet (ω -6/ ω -3, ratio 1:4).

of Th1-type antibody, BLG-IgG2a, than that in mice receiving O6H diet (Fig. 4C).

3.4 Cytokines from lymphocyte culture supernatants

Cytokines in lymphocyte culture supernatants from spleen and MLNs were measured in order to see the effect of supplemented fatty acids on BLG-induced cytokines. When splenic lymphocytes were cocultured with BLG, all sensitized mice showed significantly increased IL-4 levels (Fig. 5A) in comparison to control mice. Among the three sensitized groups,

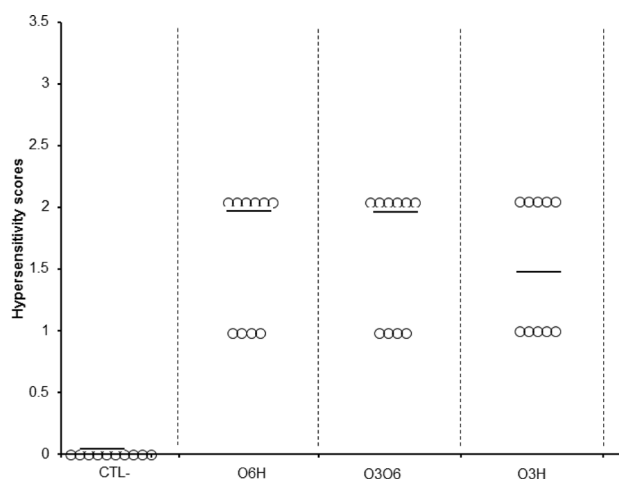


Figure 3. Hypersensitivity scores for individual mice ($n = 10$) recorded within an hour after final oral β -lactoglobulin (BLG) challenge. Each point represents an individual mouse. Median scores were shown by horizontal lines. CTL, control mice that received sham sensitization with alum in PBS; O6H, ω -6 high diet (ω -6/ ω -3, ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1); O3H, ω -3 high diet (ω -6/ ω -3, ratio 1:4).

mice that received O6H diet showed a higher IL-4 level than the other two groups. Results from MLN lymphocyte culture revealed that IL-10 and IFN- γ levels of the mice that received O6H and O3O6 diets were significantly lowered than that in mice that received O3H diet (Figs. 6C, D).

4 Discussion

We investigated whether different levels of ω -3 and ω -6 LCPUFAs in high-fat energy diets had any effect on BLG-induced immediate type hypersensitivity responses in a mouse model. We have used an energy-rich diet (4.24 kcal/g) with a high level of fat (35% of energy from fat) as a base diet to reflect nutritional composition of typical western-style diets. The three experimental diets contained different ratios of ω -6/ ω -3 (10:1

for the O6H diet, 4:1 for the O3O6 diet, and 1:1 for the O3H diet, respectively).

The allergy-preventing effects of ω -3 LCPUFA supplementation in humans were generally obtained from observations of epidemiological studies or randomized controlled studies. The power of such studies could be compromised by many factors such as the complicated nature of allergy, inclusion criteria, or lack of synchronized allergen sensitization steps in experimental subjects. Allergy is a complex disease resulting from an interaction of multiple genetic and environmental factors [25]. Therefore, mouse models such as the one in our study, using genetically identical inbred Balb/C mice, could be valuable in mechanistic and confirmatory studies.

We sensitized the mice i.p. for 3 wk with BLG and alum, the Th2-adjuvant [26]. Observation of allergen-specific serum Igs from the sera collected 3 days after the second i.p. sensitization confirmed that Th2-favoured allergic responses were established in sensitized mice (Table 4). Immune responses to BLG challenge including allergen-specific systemic immune responses (such as elevated BLG-specific serum Igs, Fig. 4), nonspecific systemic (e.g., elevated IL-4 and IFN- γ from lymphocyte culture supernatants of spleen, Fig. 5), and local immune responses (such as elevated IFN- γ , IL-12p40, and monocyte chemotactic protein 1 (MCP-1) from lymphocyte culture supernatants of MLNs, Fig. 6) were analyzed to determine allergy-reducing effects of the diets. Our results showed distinct patterns of immune responses to the BLG challenge exhibited by the mice that received three experimental diets.

The shifting of Th2 toward Th1 immune responses was observed in O3H mice. Higher concentrations of local Th1 cytokines IFN- γ and IL-12p40, chemokine MCP-1, and anti-inflammatory cytokine IL-10 from MLN of O3 mice (Figs. 6B–E), in comparison to O6H and O3O6 mice, demonstrated the ability of ω -3 LCPUFAs to suppress Th2 and induce Th1-favoured immune response. A recent asthma mouse model study using IL-12p40 $^{-/-}$ mice confirmed that IL-12p40 was essential for down-regulation of airway hyper-responsiveness [27]. MCP-1 has recently received more attention in allergy research despite the fact that its mechanism is

Table 3. Feed intake (FI) and body weight (BW) in grams

Group	Week 7		Week 8		Week 9		Week 10	
	FI	BW	FI	BW	FI	BW	FI	BW
O6H (c)	2.61 ^{b)}	19.16	2.72	19.61	2.73 ^{b)}	20.69 ^{b)}	2.61	21.41 ^{a,b)}
O3O6 (c)	2.56 ^{b)}	18.65	2.69	19.39	2.76 ^{b)}	20.51 ^{b)}	2.5	21.29 ^{a,b)}
O3H (c)	3.51 ^{a)}	19.59	2.71	20.31	3.19 ^{a)}	21.89 ^{a)}	2.55	22.12 ^{a)}
O6H	2.57 ^{b)}	19.12	2.71	19.89	2.69 ^{b)}	20.43 ^{b)}	2.33	20.5 ^{b)}
O3O6	2.5 ^{b)}	19.13	2.71	19.8	2.76 ^{b)}	20.83 ^{a,b)}	2.52	21.15 ^{a,b)}
O3H	3.58 ^{a)}	19.71	2.62	19.91	3.19 ^{a)}	21.07 ^{a,b)}	2.6	21.44 ^{a,b)}
SEM	0.12	0.41	0.25	0.37	0.09	0.39	0.22	0.39

The diet with “(c)” represents the control group for that diet.

a,b,c) Different letters in the same column designate statistically significant differences ($p \leq 0.05$).

O6H, ω -6 high diet (ω -6/ ω -3, ratio 10:1); O3O6, ω -6 and ω -3 balanced diet (ω -6/ ω -3, ratio 4:1); O3H, ω -3 high diet (ω -6/ ω -3, ratio 1:4).

Table 4. β -Lactoglobulin (BLG)-specific antibodies and total IgE in sera

Group	BLG-IgE	BLG-IgG1	BLG-IgG2a	BLG-IgG	BLG-IgA	Total-IgE
O6H (c)	ND	ND	ND	ND	ND	1585.1 ^{d)}
O3O6 (c)	ND	ND	ND	ND	ND	1639.1 ^{c,d)}
O3H (c)	ND	ND	ND	ND	ND	2126.6 ^{b)}
O6H	183.2 ^{a,b)}	17300 ^{a)}	12825 ^{b)}	45451 ^{a)}	9489.7 ^{a)}	2022.8 ^{b,c)}
O3O6	396.6 ^{a)}	16300 ^{a)}	24975 ^{a)}	64439 ^{a)}	1468.5 ^{b,c)}	2124.0 ^{b)}
O3H	412.5 ^{a)}	21665 ^{a)}	20524 ^{a,b)}	68435 ^{a)}	4474.2 ^{b)}	2796.6 ^{a)}
SEM	82.49	3979.91	3740.08	12741	1598.82	139.24

BLG-specific Igs and total IgE were determined from serum samples collected at 3 days after second dose of i.p. sensitization. Relative serum antibody concentrations (ng/mL) were obtained by interpolation from the standard curves using purified mouse antibodies (BD Pharmingen).

a,b,c) Different letters in same columns designate statistically significant differences ($p \leq 0.05$).

ND, not detectable.

still unclear. It was reported that children with resolved CMA had significantly higher concentrations of serum MCP-1 than children with persistent CMA [28]. More recently, our lab also reported the findings of higher concentrations of serum and spleen lysate MCP-1 in mice that developed protection against CMA through oral tolerance induction [29]. Accordingly, higher MCP-1 concentrations from MLNs of O3H mice indicated ω -3 LCPUFA's activity to induce MCP-1 and to inhibit allergic responses locally. Likewise, higher serum BLG-IgG2a (Fig. 4C) and lower spleen IL-4 (Fig. 5A) concentrations were observed in O3H mice, in comparison with O6H mice. These systemic immune responses lead toward Th1-favoured immune responses. However, there were no differences of the other BLG-specific serum Igs and cytokines among the three experimental groups. In general, these observations suggest that under current experimental conditions, ω -3 LCPUFAs have the ability to reduce the

severity of allergic attack by inducing Th1-favoured immune responses, but may have little or no role in preventing allergy development. Our results are in agreement with the report of Johansson et al., which found that ω -3 LCPUFA supplemented mice produced higher allergen-specific serum Ig-E and eosinophil counts in a Th2-type airway hypersensitivity mouse model [30]. Similarly, Palmer et al. recently reported that ω -3 LCPUFA did not reduce the overall incidence of Ig-E-associated allergies in the first year of life [11]. However, in contrast to our results, Yamashiro et al. revealed that mice fed with 7–9% of perilla oil (a rich source of parent ω -3 LCPUFA, ALA) had lower OVA-specific IgE [31], and attenuated intestinal mucosal damage [32]. Also, mice fed with the diet containing 7% of fish oil had lower serum anti-OVA IgE and IgG1 levels [33]. Interestingly, Korotkova et al. have studied whether dietary ratio of ω -6 to ω -3 PUFA influences the induction of immunological tolerance to OVA in neonatal rats. The group

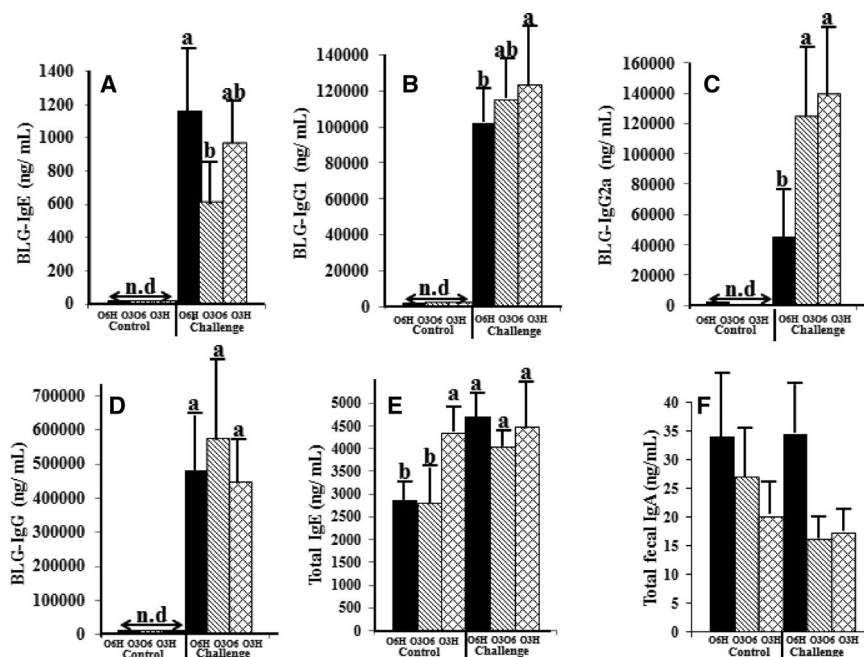


Figure 4. Allergen-specific and total antibodies. β -lactoglobulin (BLG)-specific IgE (A), IgG1 (B), IgG2a (C), and IgG (D) as well as total IgE (E) and total fecal IgA (F) antibodies were measured by ELISA. Each value represents the mean $\pm$ SEM of ten mice and different letters denote statistically significant differences ($p < 0.05$). nd, not detectable.

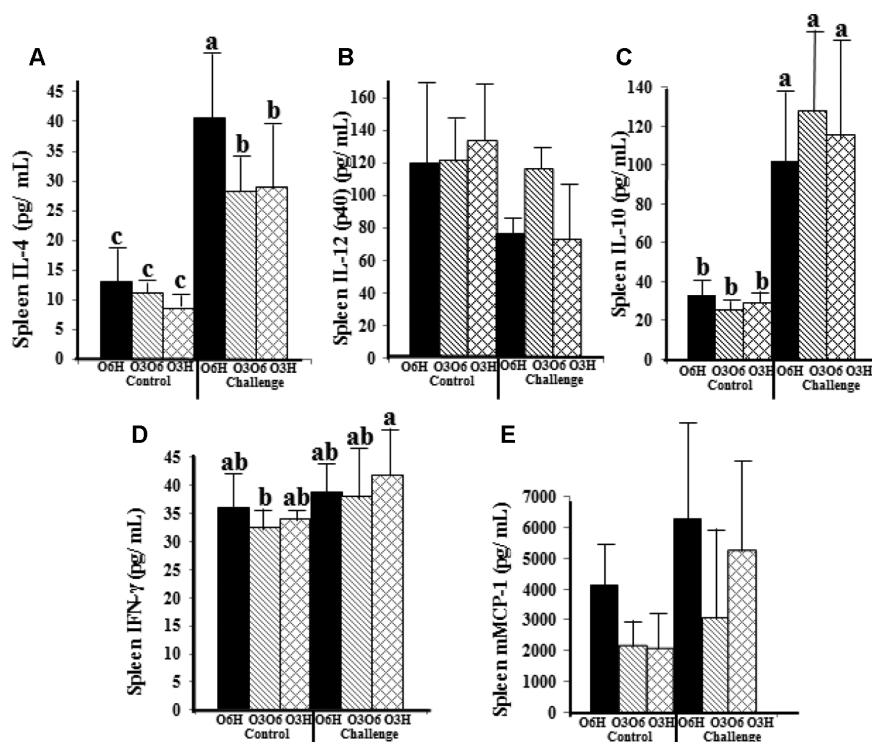


Figure 5. Cytokine profiles from the culture supernatant of spleen derived lymphocytes. Three hours after the final oral β -lactoglobulin (BLG) challenge, mice were sacrificed and lymphocytes from spleens were aseptically removed. After 72 h culture at 37°C in the presence of BLG in complete DMEM, the supernatants were collected and analyzed for IL-4 (A), IL-12p40 (B), IFN- γ (C), and MCP-1 (D). Each value represents the mean $\pm$ SEM of ten mice and different letters denote statistically significant differences ($p < 0.05$).

reported that exposure to OVA via the milk in the offspring on the ω -3 diet resulted in a lower delayed type hypersensitivity reaction (DTH) and lower OVA-specific and nonspecific antibody responses, suggesting that ω -3 PUFAs facilitated the oral tolerance induction process [34]. In comparison to the

study [34], our O3H mice had a lower serum ω -6/ ω -3 ratio (1.1 for our mice versus 2.5 for their rats), and more than two times higher EPA and DHA levels. However, we did not observe reduction of allergen-specific antibodies (Table 2). Considering the fact that we added 16.4% of oils in our

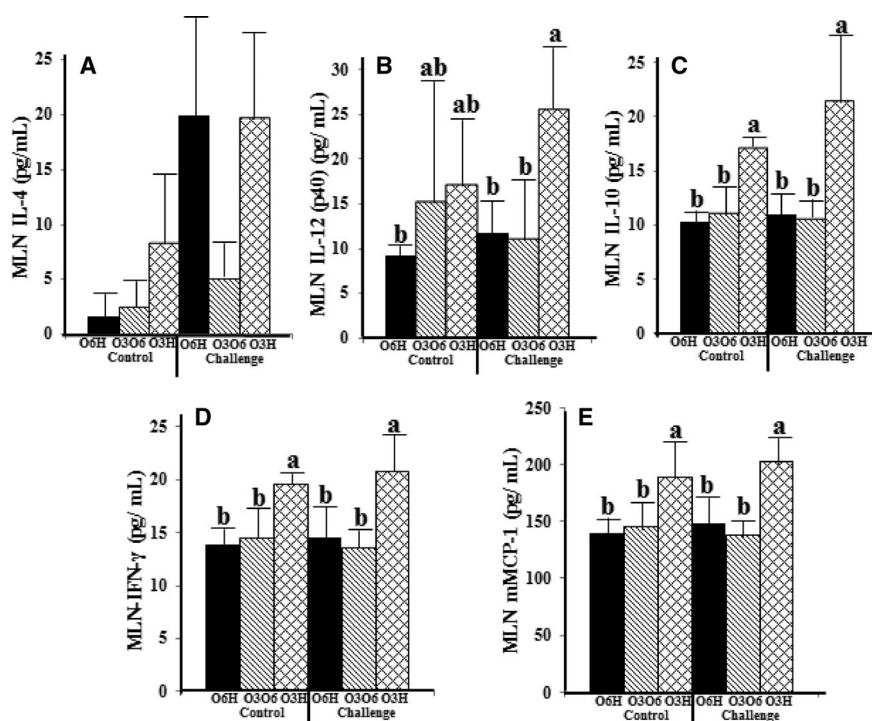


Figure 6. Cytokine profiles from the culture supernatant of lymphocytes derived from mesenteric lymph nodes (MLNs). Three hours after the final oral β -lactoglobulin (BLG) challenge, mice were sacrificed and lymphocytes from MLNs were aseptically removed. After 72 h culture at 37°C in the presence of BLG in complete DMEM, the supernatants were collected and analyzed for IL-4 (A), IL-12p40 (B), IFN- γ (C), and MCP-1 (D). Each value represents the mean $\pm$ SEM and different letters denote statistically significant differences ($p < 0.05$).

diets to reflect the fat- and energy-rich western-style diet, instead of usual 7% of perilla or linseed oil in other studies, we tempt to speculate that the fat- and energy-rich western-style diet will confer beneficial effects only when overall dietary fat content is low. This hypothesis needs to be tested. More studies are necessary to confirm the effects of ω -3 PUFAs on food allergy since recent double-blind randomized controlled trial [35], and meta-analysis studies have suggested that ω -3 or ω -3 PUFAs may not involve in primary prevention of allergic disease [36].

Mice that received the O6H diet had higher levels of local and systemic immune responses when challenged with BLG, supporting the notion that high dietary ω -6 LCPUFA promotes allergy development. Unlike O3H mice, our O6H mice revealed more pronounced Th2-mediated immune responses, such as lower concentrations of serum BLG-IgG2a (Fig. 4C), MLNs derived IL-12p40, IFN- γ , IL-10, and MCP-1 (Figs. 6B–E), and higher concentrations of spleen-derived IL-4 (Fig. 5A).

Mice that received the O3O6 diet expressed a mixed pattern of immune responses seen in O3H and O6H mice. As in O6H mice, significantly lower concentrations of IL-12p40, IFN- γ , IL-10, and MCP-1 from MLNs of O3O6 mice (Fig. 6B–E), in comparison to O3H mice, suggest the development of Th2-mediated immune response at the local level in O3O6 mice. However, the findings of higher concentrations of serum BLG-IgG2a (Fig. 4C), and lower concentrations of spleen-derived IL-4 in O3O6 mice (Fig. 5A), as in O3H mice, suggest the progress of Th1-favored immune responses at the systemic level.

An anaphylactic reaction was observed in O6H mice (Fig. 2). This was indicated by a lower rectal temperature and is caused by the sudden release of mast cell and basophil-derived mediators into the systemic circulation [37]. Interestingly, anaphylaxis development was prevented in O3O6 and O3H mice although these mice had high concentrations of serum BLG-IgE and IgG1 same as in O6H mice. Recent findings suggest that ω -3-derived lipid mediators may be responsible for anaphylaxis suppression through inhibiting degranulation of mast cells and/or reducing the potency of the released inflammatory mediators. For example, Martin et al. reported the inhibition of IgE-dependent lung mast cell degranulation from asthmatic human subjects by the lipid mediators lipoxin A4 (AA), resolvins D1, and resolvins D2 (DHA) [38]. Similarly, Rogerio et al. demonstrated the therapeutic potential of ω -3 PUFA derived D-series resolvins in allergic disorders [39]. It is well known that typical inflammatory mediators are usually ω -6 PUFA (AA) derived eicosanoids. However, oral administration of fish oil can turn AA-rich cell membrane into EPA- and DHA-rich cell membrane, and consequently produces three series of PGs and 5-series of LTs, which are less potent inflammatory mediators than two series of PGs and four series of LTs (LTB4) derived from ω -6 PUFAs. ω -6 PUFAs are essential molecules for allergic inflammation. The combination of these recent findings of ω -3 PUFAs efficacy together with the

Th1-favoured immune response seen in O3O6 and O3H mice may lead to prevention of anaphylaxis development in these groups. Taken together, these results suggest that the anaphylaxis suppression effect is a potential positive effect of ω -3 supplementation.

In accordance with our experimental setting; our results suggest that ω -3 LCPUFA offered allergy alleviation and subsequently anaphylaxis suppression effects through locally elevated IL-10 and ω -3 PUFAs derived lipid mediators, but may not prevent allergy development when BLG-sensitized mice were challenged with BLG. Our study also indicates the possibility that modulation of energy-rich western-style diet by adding ω -3 LCPUFAs may not exhibit full beneficial effects of ω -3 LCPUFAs.

This study was supported by funding from Agriculture and Agri-Food Canada to Dr. Joyce I. Boye and James McGill Professorship to Dr. Xin Zhao. The authors thank Drs. Arif Mustafa and Baurhoo Bushansingh for their help in serum fatty acid analyses. C.L.T. designed and conducted the study, acquired the data, and drafted the manuscript. J.I.B. and H.N.S. were involved in planning the study and revising the manuscript. X.Z. designed the study, interpreted the data, and revised the manuscript critically. All authors have read and approved the final manuscript.

The authors have declared no conflict of interest.

5 References

- [1] Cianferoni, A., Spergel, J. M., Food allergy: review, classification and diagnosis. *Allergol. Int.* 2009, **58**, 457–466.
- [2] Bird, J. A., Burks, A. W., Food allergy and asthma. *Prim. Care Resp. J.* 2009, **18**, 258–265.
- [3] Odermatt, A., The Western-style diet: a major risk factor for impaired kidney function and chronic kidney disease. *Am. J. Physiol. Renal Physiol.* 2011, **301**, F919–F931.
- [4] Eaton, S. B., Eaton, S. B., 3rd, Sinclair, A. J., Cordain, L. et al., Dietary intake of long-chain polyunsaturated fatty acids during the paleolithic. *World Rev. Nutr. Dietetics* 1998, **83**, 12–23.
- [5] Simopoulos, A. P., The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med. (Maywood)* 2008, **233**, 674–688.
- [6] Wall, R., Ross, R. P., Fitzgerald, G. F., Stanton, C., Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr. Rev.* 2010, **68**, 280–289.
- [7] Arita, M., Bianchini, F., Aliberti, J., Sher, A. et al., Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvins E1. *J. Exp. Med.* 2005, **201**, 713–722.
- [8] Haworth, O., Cernadas, M., Yang, R., Serhan, C. N. et al., Resolvins E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nat. Immunol.* 2008, **9**, 873–879.
- [9] Prescott, S. L., Calder, P. C., N-3 polyunsaturated fatty acids and allergic disease. *Curr. Opin. Clin. Nutr. Metab. Care* 2004, **7**, 123–129.

- [10] Furuhejm, C., Warstedt, K., Larsson, J., Fredriksson, M. et al., Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatr.* 2009, *98*, 1461–1467.
- [11] Palmer, D. J., Sullivan, T., Gold, M. S., Prescott, S. L. et al., Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial. *BMJ* 2012, *344*, e184.
- [12] Hesselmar, B., Saalman, R., Rudin, A., Adlerberth, I. et al., Early fish introduction is associated with less eczema, but not sensitization, in infants. *Acta Paediatr.* 2010, *99*, 1861–1867.
- [13] Olsen, S. F., Osterdal, M. L., Salvig, J. D., Mortensen, L. M. et al., Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am. J. Clin. Nutr.* 2008, *88*, 167–175.
- [14] Thien Francis, C. K., De Luca, S., Woods Rosalie, K., Abramson Michael, J., Dietary marine fatty acids (fish oil) for asthma in adults and children. *Cochrane Database Syst. Rev.* 2002, *2*, CD001283.
- [15] Burdge, G. C., Calder, P. C., Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 2005, *45*, 581–597.
- [16] Dunstan, J. A., Mori, T. A., Barden, A., Beilin, L. J. et al., Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J. Allerg. Clin. Immunol.* 2003, *112*, 1178–1184.
- [17] Medford, H. M., Chatham, J. C., Marsh, S. A., Chronic ingestion of a Western diet increases O-linked-beta-N-acetylglucosamine (O-GlcNAc) protein modification in the rat heart. *Life Sci.* 2012, *90*, 883–888.
- [18] Marsh, S. A., Dell'italia, L. J., Chatham, J. C., Interaction of diet and diabetes on cardiovascular function in rats. *Am. J. Physiol. Heart Circulat. Physiol.* 2009, *296*, H282–H292.
- [19] Fritsche, K. L., Johnston, P. V., Rapid autoxidation of fish oil in diets without added antioxidants. *J. Nutr.* 1988, *118*, 425–426.
- [20] Schouten, B., van Esch, B. C., Hofman, G. A., van Doorn, S. A. et al., Cow milk allergy symptoms are reduced in mice fed dietary synbiotics during oral sensitization with whey. *J. Nutr.* 2009, *139*, 1398–1403.
- [21] Thang, C. L., Baurhoo, B., Boye, J. I., Simpson, B. K. et al., Effects of *Lactobacillus rhamnosus* GG supplementation on cow's milk allergy in a mouse model. *Allerg. Asthma Clin. Immunol.* 2011, *7*, 20.
- [22] Adel-Patient, K., Ah-Leung, S., Creminon, C., Nouaille, S. et al., Oral administration of recombinant *Lactococcus lactis* expressing bovine β -lactoglobulin partially prevents mice from sensitization. *Clin. Exp. Allergy* 2005, *35*, 539–546.
- [23] Su, C. W., Cao, Y., Kaplan, J., Zhang, M. et al., Duodenal helminth infection alters barrier function of the colonic epithelium via adaptive immune activation. *Infection Immunity* 2011, *79*, 2285–2294.
- [24] Glaser, C., Demmelmaier, H., Koletzko, B., High-throughput analysis of total plasma fatty acid composition with direct in situ transesterification. *PLoS One* 2010, *5*, e12045.
- [25] Holloway, J. W., Yang, I. A., Holgate, S. T., Genetics of allergic disease. *J. Allergy Clin. Immunol.* 2010, *125*, S81–S94.
- [26] Lindblad, E. B., Aluminium adjuvants—in retrospect and prospect. *Vaccine* 2004, *22*, 3658–3668.
- [27] Onari, Y., Yokoyama, A., Haruta, Y., Nakashima, T. et al., IL-12p40 is essential for the down-regulation of airway hyperresponsiveness in a mouse model of bronchial asthma with prolonged antigen exposure. *Clin. Exp. Allergy* 2009, *39*, 290–298.
- [28] Glez, P. P., Franco, Y. B., Matheu, V., MIP-1 α , MCP-1, and desensitization in anaphylaxis from cow's milk. *N. Engl. J. Med.* 2012, *367*, 282–284.
- [29] Thang, C. L., Boye, J. I., Zhao, X., Low doses of allergen and probiotic supplementation separately or in combination alleviate allergic reactions to cow β -lactoglobulin in mice. *J. Nutr.* 2013, *143*, 136–141.
- [30] Johansson, S., Lonnqvist, A., Ostman, S., Sandberg, A. S. et al., Long-chain polyunsaturated fatty acids are consumed during allergic inflammation and affect T helper type 1 (Th1)- and Th2-mediated hypersensitivity differently. *Clin. Exp. Immunol.* 2010, *160*, 411–419.
- [31] Watanabe, S., Sakai, N., Yasui, Y., Kimura, Y. et al., A high alpha-linolenate diet suppresses antigen-induced immunoglobulin E response and anaphylactic shock in mice. *J. Nutr.* 1994, *124*, 1566–1573.
- [32] Yamashiro, Y., Ohtsuka, Y., Yabuta, K., The regulation of intestinal hypersensitivity reactions to ovalbumin by ω -3 fatty acid enriched diet: studies of IEL and LPL in mucosal damage. *Pediatrics Int.* 1994, *36*, 550–556.
- [33] de Matos, O. G., Amaral, S. S., Pereira da Silva, P. E., Perez, D. A. et al., Dietary supplementation with omega-3-PUFA-rich fish oil reduces signs of food allergy in ovalbumin-sensitized mice. *Clin. Dev. Immunol.* 2012, *2012*, 236564.
- [34] Korotkova, M., Telemo, E., Yamashiro, Y., Hanson, L. A. et al., The ratio of n-6 to n-3 fatty acids in maternal diet influences the induction of neonatal immunological tolerance to ovalbumin. *Clin. Exp. Immunol.* 2004, *137*, 237–244.
- [35] D'Vaz, N., Meldrum, S. J., Dunstan, J. A., Martino, D. et al., Postnatal fish oil supplementation in high-risk infants to prevent allergy: randomized controlled trial. *Pediatrics* 2012, *130*, 674–682.
- [36] Anandan, C., Nurmatov, U., Sheikh, A., Omega 3 and 6 oils for primary prevention of allergic disease: systematic review and meta-analysis. *Allergy* 2009, *64*, 840–848.
- [37] Khan, B. Q., Kemp, S. F., Pathophysiology of anaphylaxis. *Curr. Opin. Allergy Clin. Immunol.* 2011, *11*, 319–325.
- [38] Martin, N., Ruddick, A., Arthur, G. K., Wan, H. et al., Primary human airway epithelial cell-dependent inhibition of human lung mast cell degranulation. *PLoS One* 2012, *7*, e43545.
- [39] Rogerio, A. P., Haworth, O., Croze, R., Oh, S. F. et al., Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. *J. Immunol.* 2012, *189*, 1983–1991.