

Effect of dietary fat on skin reactivity against histamine, Th1 and Th2 cytokine levels and some serum parameters in mice

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Abstract

Changes in diet may be associated with the increase in allergic disease; change to high-calorie and high-fat diets may be a factor. In this study our objective was to determine skin reactivity of histamine and serum cytokine concentrations in mice fed diets containing different amounts of fat. Histamine reactivity was performed on mice back skin and serum cytokine concentrations were measured by ELISA in mice injected with anti-CD3 antibody. We measured serum interferon- γ as a Th1-type cytokine and interleukin-4 as a Th2-type cytokine. Mice fed a high fat diet displayed enhanced skin reactivity of histamine and higher IL-4 levels in serum. These data suggest that a high fat diet may play a role in enhancing allergic reactions.

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Introduction

Allergic diseases, such as food allergy, atopic dermatitis, eczema, asthma, etc., appear to be on the increase (Bloebaum et al., 2004). It is thought that this increased incidence is related to a change of diet (Chinn, 2003; Marshall, 2004; Spector and Surette, 2003). Black and Sharpe (1997) proposed that there is a connection between dietary fat and asthma, with dietary linoleic acid and immunoregulatory prostaglandin E₂ production being linked. However, there is little information about the relationship between nutrients (including fat)

and the vascular permeability response in allergy. Histamine, as a chemical mediator of the allergic reaction, is the most important factor, especially in atopic dermatitis (McMillan et al., 1985). Mast cells release histamine from granules in the cytosol when they are stimulated by allergen. The histamine released mediates inflammatory reactions, such as edema or redness. The Th1 and Th2 cytokine balance has been proposed as a concept for predisposition to several diseases such as autoimmune disease, allergy or diabetes (Fuse et al., 2003; Haggqvist and Hultman, 2003; Jolly and Fernandes, 1999; Kleemann et al., 1998; Matsushima et al., 2003; Suzuki et al., 2003). It is suggested that feeding a high fat diet for long periods of time changes the constitution of immunologic reactions. Harbige (2003) reviewed the effects of fatty acids, especially n-6 fatty acids and the n-6/n-3 balance, on the Th1 and Th2

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immune response and autoimmunity, and concluded that the effects of some fatty acids on Th2 responses were unclear. Furthermore, information on the effect of the quantity of dietary fat on the Th1 versus Th2 balance and on the response to histamine in the skin is lacking. Therefore, in this investigation we studied the effect of the amount of fat in the mouse diet on skin reactivity, and on serum cytokine (interferon- γ [IFN- γ] and interleukin-4 [IL-4]), IgG2, IgG1, and IgE concentrations.

Materials and methods

Materials

Bovine serum albumin (BSA, Fraction V) and fluorescein isothiocyanate were purchased from Sigma Chemical Co., St Louis, MO, USA; histamine from Wako Pure Chemical Co., Osaka, Japan; anti-CD3 ϵ antibody from BD Bioscience Pharmingen Co., San Diego, CA, USA; and hamster IgG (Capple product) from ICN Pharmaceuticals Inc., Aurora, OH, USA.

ELISA kits for IFN- γ and IL-4 were purchased from R&D Systems Inc., Minneapolis, MN, USA; mouse immunoglobulin ELISA kits from Bethyl Co. Ltd., Montgomery, TX, USA.

Animals

Male ICR strain mice (Charles River Japan Co. Ltd., Yokohama, Japan), weighing 30–45 g, were used. These animals were kept in an environmentally controlled room ($25 \pm 3^\circ\text{C}$, $50 \pm 10\%$ humidity) with a light:dark cycle of 12 h and allowed free access to water. Mice were housed together in groups of three animals in standard plastic mouse cages ($30 \times 20 \times 13\text{ cm}^3$). Coprophagy was not observed. The mice were maintained and handled according to the recommendations of the experimental animal committee of the National Food Research Institute.

Diet and equipment

Mice were fed water and diets containing different amounts of fat (Table 1) ad libitum, and weighed at 2–3 day intervals. The fat contents were from 6% to 24% (w/w) and soybean oil was the fat source. The amounts of the carbohydrate sources were proportionally reduced with the increasing amounts of soybean oil in the diets. The soybean oil (Kanto Chemical Co., Tokyo, Japan) consisted of linoleic acid (51–57%), oleic acid (32–36%), stearic acid (4.7–7.3%), and palmitic acid (2.4–6.8%). Energy contents of the diets were increased in proportion with the fat content. Dietary intakes of

Table 1. Content of diets differing in fat content (g/kg)

Ingredient	Diet content (g/kg)			
	Fat 6%	Fat 12%	Fat 18%	Fat 24%
Whole egg protein	160	160	160	160
Corn starch	431	393	355	317
α -corn starch	143	130	118	105
Soybean oil	60	120	180	240
Sucrose	108	99	89	79
Cellulose	50	50	50	50
Salt mix (AIN93G)	35	35	35	35
Vitamin mix (AIN93G)	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
Gross energy (kcal/kg)	3910	4210	4510	4810

each group were monitored with Roden Café® (Oriental Yeast Co. Ltd., Tokyo, Japan), which was feedbox with cover, at 2–3 day intervals.

Vascular permeability assay

Skin test for histamine reactivity of mouse dorsal skin was performed using our previously reported method (Yamaki et al., 2002). Briefly, under light ether anesthesia, male ICR mice were injected with fluorescence isothiocyanate labeled BSA (F-BSA) into the tail vein as a tracer of vascular permeability. Immediately after the F-BSA injection, histamine (0.05 ml), to induce vascular permeability, was injected intradermally into six points in the shaved dorsal skin of the mice. Doses of histamine used were in the range 0.16–2.50 $\mu\text{g}/\text{site}$ for mice. After 30 min, the mice were sacrificed under light ether anesthesia by removing the blood, and skin samples were cut to a size of 10 mm in diameter. Blood was collected for measuring F-BSA in serum. Each circular skin sample was put directly into a well of a 24-well tissue culture plate (Falcon, 3047). One milliliter of formamide was added to the well. The plate was incubated for 2 h at 50°C . The fluorescent intensity of the skin samples was then measured automatically using a fluorescent plate reader with 485 nm for excitation and 530 nm for emission.

The total amount of dye was calculated by means of a standard calibration curve.

Measurements of cytokines and immunoglobulins in serum

Anti-CD3 ϵ (2.5 $\mu\text{g}/\text{mouse}$) was injected into the tail vein and 2 h before blood was collected by cardiac puncture. Cytokine levels (IFN- γ as Th1-type and IL-4 as Th2-type) in mouse serum were measured by ELISA

(R&D Systems Inc., McKinley Place, NE, USA). Immunoglobulin (IgG2a, IgG1 and IgE) levels in serum were measured by ELISA (Bethyl Lab. Inc., Montgomery, TX, USA).

Statistics

Statistical comparisons between groups were performed using the one-way ANOVA followed by the Bonferroni correction for multiple comparison. Differences among means were considered significant when $p < 0.05$.

Results

Weight gain

Fig. 1 shows the body weight gain and calorie intake in mice fed diets containing 6–24% fat for 3 months. Six percent fat fed mice showed greater weight gain than the other groups. The body weights of mice fed 12–24% fat were not different, but after 2 months the order of the groups is the same as that of the fat content.

Fig. 1B shows calorie intakes over time in mice fed 6–24% fat diets for 3 months. Calorie intake per mouse per day was not significantly different among the different groups.

Fig. 1C shows food intake over time in mice given 6–24% fat content diets for 3 months. Food intake per mouse per day was not significantly different among the different groups.

Change of skin reactivity against histamine

Vascular permeability of skin upon histamine challenge was measured in mice fed for 4, 8 or 12 weeks on diets containing 6–24% fat. Histamine-induced vascular permeability increased in a dose-dependent manner up until 1.25 $\mu\text{g}/\text{site}$. After 4 weeks of feeding, vascular permeability in the 6% and 12% groups was higher than in the 18% and 24% groups (data not shown). Furthermore, at 8 weeks feeding reactivity to histamine in the 18% and 24% groups increased, and these groups were almost the same as the other groups (data not shown).

Fig. 2A shows vascular permeability change on histamine challenge after 12 weeks feeding of 6–24% fat diets. The mice fed the highest fat diet (24% fat) had a significantly increased response to histamine compared with the lowest fat fed mice at 0.16 and 0.63 $\mu\text{g}/\text{site}$ histamine. The response in the 6% and 12% groups displayed little change from 1 to 3 months. On the other hand, for the 18% and 24% groups, the response increased from 1 to 3 months (data not shown). The 3

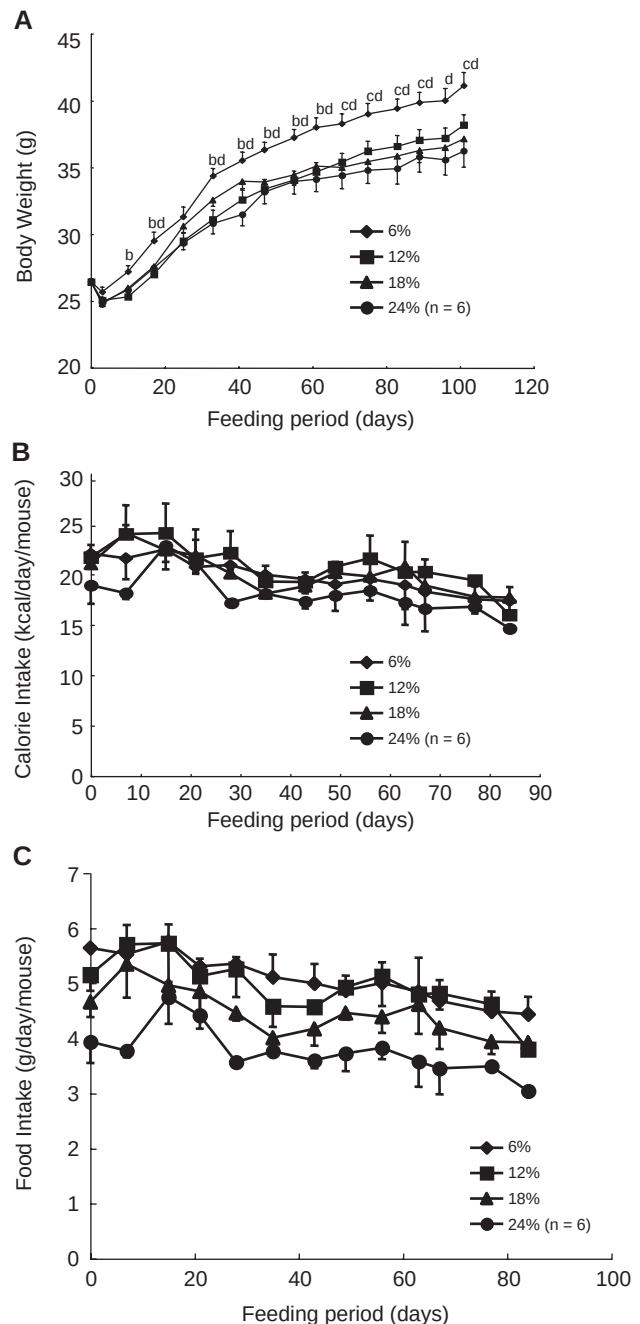


Fig. 1. Body weight and calorie and food intakes of male ICR mice fed diets differing in fat content for 3 months. Male ICR strain mice were fed diets with different fat contents (6%, 12%, 18%, and 24% fat) for approximately 3 months. In these periods, the body weight (A), calorie intake (B), and food intake (C) were measured continuously at 2 or 3 day intervals. Calorie intake was calculated from the amount of diet remaining in a cage. $b = p < 0.05$, vs. corresponding 12% fat-fed group. $c = p < 0.05$, vs. corresponding 18% fat-fed group. $d = p < 0.05$, vs. corresponding 12% fat-fed group.

months data suggested that a high fat diet may enhance allergic reactions. Fig. 2B shows the slopes of histamine response in mouse dorsal skin. The slope was extracted

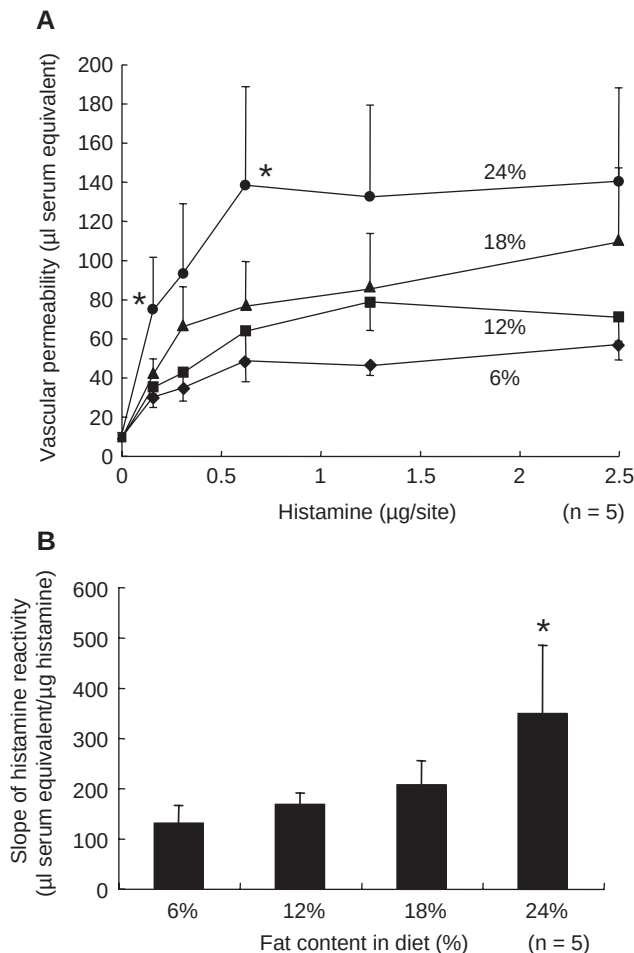


Fig. 2. The skin reactivity against histamine (A) in male ICR mice fed diets differing in fat content for 3 months and slopes of histamine response (B) in range from 0 to 0.16 μg/site. Male ICR strain mice were measured for skin reactivity against histamine as vascular permeability increase after 12 weeks feeding of diets with different fat contents. The skin reactivity against histamine was measured by using fluorescent-labeled BSA as a tracer of vascular permeability. Data are expressed as means and standard errors of means. * $p < 0.05$, vs. corresponding 6% diet group.

from the formula:

$$\text{Slope} = ((\text{vascular permeability to } 156 \text{ ng histamine}) - (\text{vascular permeability to vehicle}))/0.156).$$

The slope value was used as a marker for the allergic reaction (Dreborg, 2001; Dreborg et al., 1987). The high fat diet (24%) increased the histamine response in mouse dorsal skin significantly compared with the 6% fat diet.

Cytokine levels of Th1- and Th2-type in serum after injection of CD3 antibody

Fig. 3 shows the concentrations of Th1 and Th2 cytokines in the serum of mice. The serum level of

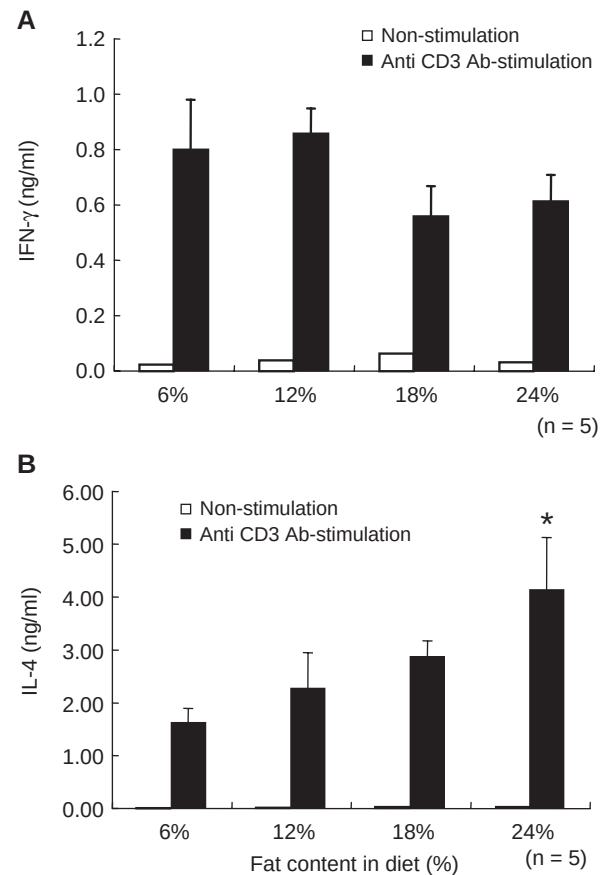


Fig. 3. Interferon-γ and interleukin-4 levels in serum of ICR strain mice stimulated by anti-CD3 antibody injection. Mice were injected with anti-CD3 antibody intravenously. After 2 h, blood was collected by cardiac puncture. Interferon-γ (A) and interleukin-4 (B) were measured by ELISA. Non-stimulation serum was collected after injection of the same subclass of hamster immunoglobulin. Values are expressed as mean $\pm$ standard error. * $p < 0.05$, vs. corresponding 6% diet group.

cytokines were very low in unstimulated mice. However detectable levels of cytokines in serum were observed when mice were injected with the anti-CD3 antibody intravenously. The serum levels of IFN-γ decreased with increasing fat content of the diets (Fig. 3A) but were not statistically significant. In contrast, IL-4 level in serum increased with increasing fat content of the diets (Fig. 3B). The 24% fat diet shifted the Th1/Th2 balance towards a Th2 response compared with the lower fat diets.

Immunoglobulin levels in serum

Fig. 4 shows the concentrations of IgG and IgE in the mouse sera. The subclass IgG1, a Th2-type immunoglobulin, displayed almost no change. IgE, another Th2-type immunoglobulin, had a tendency to increase with fat content of the diet. On the other hand, IgG2a,

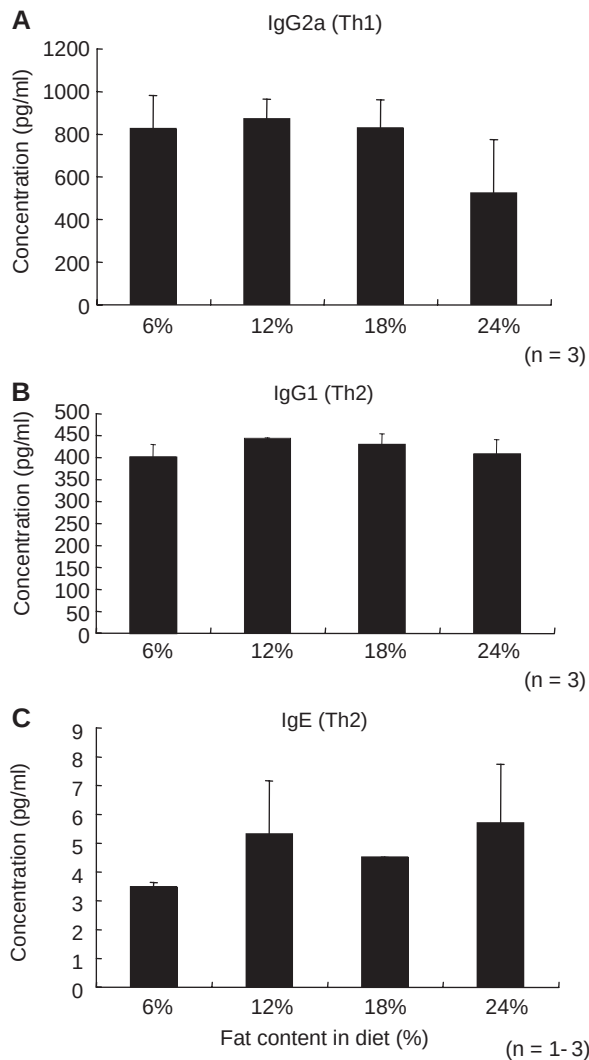


Fig. 4. Immunoglobulin levels in serum of ICR strain mice fed diets differing in fat content for 3 months. Immunoglobulin subtype levels, IgG2a (Th1-type, A), IgG1 (Th2-type, B), and IgE (Th2-type, C), in mouse serum were measured by ELISA. Values are expressed as mean $\pm$ standard error.

a Th1-type immunoglobulin, tended to decrease in the serum of mice fed 24% fat-diet. IgG2b and IgG3 displayed no change (data not shown). These results suggest that a high fat diet shifts the Th1/Th2 balance towards the Th2 response type.

Discussion

We measured skin reactivity against histamine and serum levels of IFN- γ as a Th1-type cytokine and IL-4 as a Th2-type cytokine in mice in order to investigate the effect of the fat content of the diet. The mice fed the lowest fat diet had an increased body weight compared

to the other groups over the course of the experiment; however there was no difference between the groups in calorie intake. In this study carbohydrate was used to adjust for the different amounts of fat used. Therefore, the mice fed low fat consumed more carbohydrate. These data indicate that carbohydrate has more activity for increasing body weight than fat.

Reactivity to histamine in the mouse dorsal skin increased depending on the fat content of the diet. Also, the slope values of the histamine response (from 0 to 0.16 μ g/site) increased depending on the fat content of the diet. This result suggests that the fat content of the diet is involved in sensitivity to histamine in the mouse skin. In general, dietary fatty acid composition is reflected in cell membrane lipid composition, including that of lymphocytes (Avula et al., 1999). Injected histamine binds to the histamine receptor on endothelial cells of venules in the local area. After that shape change of the endothelial cells occurs, and vascular permeability increases and edema is formed. It is possible that endothelial cell membrane lipid composition and contents are changed by diet. In order to confirm this, analysis of the fatty acid composition of the endothelial cells is required.

The role of the Th1/Th2 balance as a biological marker is now well known (Decker et al., 1996; Muller et al., 1998; Nakamura et al., 1997; Xu et al., 1999). Th1 cytokines are dominant in patients with graft versus host disease (GVHD) or diabetes. On the other hand, Th2 cytokines are dominant in patients with allergy. Th1-type helper T cells produce IFN- γ and IL-2, and Th2-type helper T cells produce IL-4, 5, 6 and 10, etc. Therefore, the relative concentrations of cytokines released clarify which type of Th cell is dominant. The serum level of IL-4 in mice fed a high fat diet was higher than those fed a low fat diet. On the other hand, the serum level of IFN- γ was the opposite. This means that the Th1/Th2 balance shifted towards Th2, which is more pro-allergic. Previous experiments used several different fats (not purified soybean oil) in the diet, we demonstrated that high fat (linoleic acid rich) diet fed mice shifted to Th2 response. However, the next question is which factor is important. For example, it may be the general level of total fat intake, the level of linoleic acid specifically (from soybean) or the shift to a lower carbohydrate level in the high fat diet. Unfortunately, in order to clarify the factors we need more experiments on the fats and calorie intake. Especially experiments using more purified fatty acids or experiments using calorie controlled diets are required for clear results. Although many papers report cytokine production from isolated lymphocytes stimulated in vitro, there is a possibility that this may not reflect the real cytokine balance in vivo. Thus, we made direct measurements of cytokines in serum of mice activated by injection of anti-CD3 ϵ , that activates helper T cells. We successfully measured

Th1/Th2 cytokine balance in vivo. Also serum immunoglobulin levels displayed a similar tendency, towards the more allergic Th2-type. These results clearly indicate that a high fat diet shifted to Th2-type dominant serum markers. Some other reports state that fatty acid or high fat diet changes the Th1/Th2 balance (Jolly and Fernandes, 1999; Kleemann et al., 1998; Switzer et al., 2003; Wallace et al., 2001).

A low fat and low calorie diet enhanced the delayed type hypersensitivity reaction in humans (Santos et al., 2003). Recently Han et al. (2003) reported that a low fat diet (28% energy as fat) enhanced T-cell mediated immune functions (delayed type hypersensitivity) in older adults. Thus, in humans lowering the amount of fat in the diet may increase Th1-type responses, which is in agreement with our results, although we used fat contents in the range of 6–24% fat by weight (12–48% energy as fat).

Mito et al. (2002) reported the effects of diet-induced obesity on immune reactions in mice. In obese mice, OVA sensitization increased mast cell numbers in the tracheal mucosa. This means that overweight mice have a high sensitivity to antigens in trachea. We did not measure mast cell numbers, but there may be a possibility that mast cell number increased in addition to reactivity to histamine in skin.

The oil used in these experiments was soybean oil, which is about 50% linoleic acid (n-6 fatty acid) and 8% α -linolenic acid (n-3 fatty acid). There are some reports that n-3 fatty acids induce T cell apoptosis (Avula et al., 1999; Switzer et al., 2003). However, the effect of a high fat diet on Th1/Th2 cytokine balance is not explained from this fact. It is worth noting that n-3 fatty acids have an immunomodulating effect on helper T lymphocytes. For example, one study reports a clear shift in the Th1/Th2 cytokine mRNA ratio towards Th2 in the gut-associated immune system in rats fed fish oil (Kleemann et al., 1998). Furthermore, Wallace et al. (2001) reported that n-3 fatty acids act to inhibit production of Th1-type cytokines with little effect on Th2 cytokines. We did not examine the effects of changing the type of fatty acid in the diet, but this should be examined in our model in the future.

Our study is one of the few on the effects of the amount of fat in the diet and the allergic condition using in vivo data. From the results, it appears that a high fat diet shifted the immune phenotype towards Th2-type. Therefore we suggest that a high fat diet may have an enhancing effect on allergic disease.

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