Dietary oxidized oil influences the levels of type 2 T-helper cell-related antibody and inflammatory mediators in mice

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The aim of this present study was to investigate the effect of amount and degree of oxidation of dietary oil on type 2 T-helper cell (TH)-related immune responses. Four groups of BALB/c mice were fed either 50 g soyabean oil/kg (50-S), 50 g oxidized oil/kg (50-O), 150 g soyabean oil/kg (150-S) or 150 g oxidized oil/kg (150-O). After 14 weeks consuming the experimental diets, the mice were immunized with ovalbumin (OVA) plus Al and antigen-specific immunoglobulin (Ig)E, IgG1 and IgG2a, inflammatory mediators such as prostaglandin (PG) E2 and leukotriene (LT)B4 were determined. Higher hepatic microsomal cytochrome P450 was noted in mice fed 150 g oxidized oil/kg compared with those of other groups. OVA-specific IgG1 and IgE were higher in mice fed 150 g oxidized oil/kg compared with those of the other groups. The data suggested the interleukin (IL)-4: interferon (IFN)-γ ratio was higher in mice fed 50 g dietary oxidized oil/kg compared with that of the 50-S group. The IL-5:IFN-γ ratios were higher in the 150-S and 150-O groups than in the 50-S and 50-O groups. PGE2 and LTB4 produced by macrophages stimulated by lipopolysaccharide were highest in mice in the 150 g oxidized oil/kg group. The data suggested that an increased intake of oxidized oil might exert an unfavourable effect on the TH2 response involved in allergic disease.

Oxidized oil: Immunoglobulin E: Asthma: Prostaglandin E2: Leukotrienes

It has been well documented that environmental factors such as polluted air, tobacco, diet and infectious diseases play a critical role in the recently increasing prevalence of allergic diseases (Kimber, 1998). Several studies have demonstrated that both the quantity and quality of fat intake may play an important role in increasing the prevalence and severity of allergic diseases (Black & Sharpe, 1997; Hodge et al. 1998). Certain diets such as low-fat and fish-oil-containing diets have been shown to modulate the disease course of asthma or chronic lung disease (Arm et al. 1988; Smit et al. 1999; Schwartz, 2000).

Although the mechanism explaining how dietary fat affects immune response is still not well defined, several studies have suggested the involvement of a malfunction of macrophages and alterations in the production of proinflammatory mediators such as arachidonic acid, leukotrienes (LT), platelet activating factor, interleukin (IL)-1 and tumour necrosis factor-α (Sperling et al. 1987; Endres et al. 1989). In particular, the amount and composition of polyunsaturated fatty acids have been found to affect the synthesis of immune mediators such as prostaglandins (PG) and LT (Caughey et al. 1996). Much attention has been focused on polyunsaturated fatty acids of the n-3 class such as docosahexaenoic acid and eicosapentanoic acid, which are suggested to decrease the formation of these inflammatory mediators, including LTB4, LTC4, LTD4 and LTE4. Very few papers concerning the effect of oxidized oil by frying on immune responses have been reported; however, dietary oxidized oil may actually play an important role in health and in diseases (Lin et al. 1997). Dietary oxidized oil influences the hepatic and serum levels of triacylglycerol, cholesterol and hepatic cytochrome P450 (Lai & Lin, 1997; Lai et al. 1997). In addition, the degree of lipid oxidation can affect lymphoid organs and influence immune responses in normal mice (Oarada et al. 1989, 1991). It is believed that an increased dietary content of oxidized oil

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; LT, leukotriene; OVA, ovalbumin; PG, prostaglandin; TH, T-helper cell.

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might result in the aggravation of such immune diseases as autoimmune diseases, allergic diseases and tumours (Lin et al. 1996).

No studies concerning the effect of oxidized oil on the immune response in animal models of allergic disease have been documented. This present study further investigated the effect of oxidized oil on serum levels of antigen-specific antibody, cytokine production pattern and inflammatory mediators.

Materials and methods

Preparation of oxidized oil and diets

The oxidized oil was prepared as follows: 5.5 g soyabean oil (President Co., Tainan, Taiwan) was poured into a cast-iron wok (40 cm internal diameter, 10 cm central depth, 6.5 litre capacity) and heated on a gas stove which was adjusted to maintain the oil temperature at 205 ± 5°C. Wheat-flour dough sheets (12 × 4.5 × 0.15 cm, about 11 g in weight) were fried in the oil, one at a time. The wheat-flour dough was made by mixing together (g): high-gluten wheat flour 1500, table sugar 200, baking powder 5, water 600. The frying proceeded for 6 h/d and was repeated successively for 4 d according to the previously reported procedures (Huang et al. 1988; Lin et al. 1996). The resultant oxidized oil as well as the unfried fresh soyabean oil was stored at −20°C for the preparation of test diets. The fatty acid compositions of the fresh soyabean oil and the oxidized oil were measured according to the method described by Lee et al. (1990). The degree of oxidation was evaluated as acid value, absorbance at 233 nm, total polar compounds, and the non-urea-adductable fractions (Sallee, 1971). The quality of the soyabean oil declined after 24 h frying process (Table 1). The composition of experimental diets is summarized in Table 2. The casein content was increased in the 150 g oil/kg diets to provide the same % energy from protein as the 50 g oil/kg diet.

Animals and immunization

Female BALB/c mice between 6 and 8 weeks of age were purchased from the Animal Centre of the College of Medicine at National Taiwan University. The animal room had a 12 h light–dark cycle and a constant temperature (25 ± 2°C) and humidity. The mice were housed individually in stainless-steel wire cages and fed on a non-purified diet (Lab Rodent Chow; Ralson Purina, St Louis, MO, USA) before being fed on the experimental diets. Animal care and handling conformed to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (National Research Council, 1985). Each mouse was fed on the experimental diet starting from 13-weeks-old. The four diets used contained either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S) or 150 g oxidized soyabean oil/kg (150-O). The mice were given free access to the test diets throughout the experiment. The mice were weighed twice per week, and food consumption was measured every 2–3 d. Each group included eight mice. In addition, eight mice per group were killed for lipid analysis, cytokine assay and proliferative study.

After 14 weeks of consuming the test diets (27-weeks-old), four groups of BALB/c mice were immunized by an intraperitoneal injection of 0.1 ml PBS solution containing ovalbumin (OVA, 2 μg) with AlOH as the adjuvant. The mice were immunized again, 2 weeks later, with 6 μg OVA plus the same adjuvant (Chuang et al. 1996). Blood was obtained at days 0, 14 and 28 from the retro-orbital venous plexus and centrifuged at 12 000 g for 10 min. Serum was collected and stored at −20°C before further assay.

Determination of serum anti-ovalbumin antibody levels

Serum anti-OVA IgE, IgG1 and IgG2a antibody titers were measured by ELISA. Briefly, ninety-six-well flat-bottomed microtiter plates (Costar, Cambridge, MA, USA) were coated with 10 μg OVA/ml NaHCO3 buffer, pH 9.6. After overnight incubation at 4°C, the plates were washed three times with PBS and coated with 100 μl PBS solution containing 2 μg/ml OVA in PBS. The wells were incubated for 1 h at 37°C and washed three times with PBS. The wells were blocked with 100 μl blocking solution (PBS solution containing 3% w/v bovine serum albumin) at 37°C for 1 h. Then, 100 μl of various dilutions of serum samples were added to each well and incubated at 37°C for 1 h. After washing, 100 μl peroxidase-conjugated goat anti-mouse serum was added to each well and incubated at 37°C for 1 h. After washing, 100 μl PBS solution containing 0.2% w/v o-phenylenediamine dihydrochloride and 0.01% w/v H2O2 in 50 mm Na2CO3 buffer, pH 7.4 were added to each well and incubated at 37°C for 30 min. The reaction was stopped by the addition of 100 μl 2 N H2SO4. Absorption at 492 nm was measured by ELISA. Briefly, ninety-six-well flat-bottomed microtiter plates (Costar, Cambridge, MA, USA) were coated with 10 μg OVA/ml NaHCO3 buffer, pH 9.6. After overnight incubation at 4°C, the plates were washed three times with PBS and coated with 100 μl PBS solution containing 2 μg/ml OVA in PBS. The wells were incubated for 1 h at 37°C and washed three times with PBS. The wells were blocked with 100 μl blocking solution (PBS solution containing 3% w/v bovine serum albumin) at 37°C for 1 h. Then, 100 μl peroxidase-conjugated goat anti-mouse serum was added to each well and incubated at 37°C for 1 h. After washing, 100 μl PBS solution containing 0.2% w/v o-phenylenediamine dihydrochloride and 0.01% w/v H2O2 in 50 mm Na2CO3 buffer, pH 7.4 were added to each well and incubated at 37°C for 30 min. The reaction was stopped by the addition of 100 μl 2 N H2SO4. Absorption at 492 nm was measured by ELISA.

Table 1. The fatty acid composition (g/100 g total fatty acids) and the degree of oxidation of fresh soyabean oil and oxidized frying soyabean oil

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>50-S</th>
<th>50-O</th>
<th>150-S</th>
<th>150-O</th>
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<tbody>
<tr>
<td>Fresh soyabean oil</td>
<td>50</td>
<td>–</td>
<td>150</td>
<td>–</td>
</tr>
<tr>
<td>Fried soyabean oil</td>
<td>–</td>
<td>50</td>
<td>–</td>
<td>150</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>224</td>
<td>224</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>325</td>
<td>325</td>
<td>258</td>
<td>258</td>
</tr>
<tr>
<td>Sucrose</td>
<td>325</td>
<td>325</td>
<td>258</td>
<td>258</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>AIN-76 vitamin mix†</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>AIN-76 mineral mix†</td>
<td>35</td>
<td>35</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Energy (kJ/kg)</td>
<td>16170</td>
<td>16170</td>
<td>18102</td>
<td>18102</td>
</tr>
<tr>
<td>% Energy from protein</td>
<td>20.8</td>
<td>20.8</td>
<td>20.8</td>
<td>20.8</td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>11.7</td>
<td>11.7</td>
<td>31.3</td>
<td>31.3</td>
</tr>
</tbody>
</table>

* Ingredient sources: casein, methionine and choline, Sigma Chemical (St Louis, MO, USA); sucrose, Taiwan Sugar Company (Taipei, Taiwan); cornstarch, Roquatte (Paris, France); α-cellulose, ARBOCEL®; type BE 600/300, J. Bettenmaier & Söhne (Eilwalden-Holzmühle, Germany).
† 50-S: 50 g fresh soyabean oil/kg diet; 50-O: 50 g oxidised soyabean oil/kg diet; 150-S: 150 g fresh soyabean oil/kg diet; 150-O: 150 g oxidised oil/kg diet. For details of oxidation procedure, see p. 912.
Spleen cells, at a concentration of 5 \times 10^6 cells, were collected from OVA-immunized mice with strong proliferative response further, spleen cells were plated in ninety-six-well round-bottomed plates with a concentration of 1 \times 10^6 cells/ml in RPMI 1640 medium supplemented with 20 ml defined serum replacement/l (TCM\textsuperscript{TM}; Celox Co., Hopkins, MN, USA), 4 mM L-glutamine, 25 mM-HEPES, 5 \times 10^{-5} M 2-mercaptoethanol, 100 U penicillin/ml, 100 \mu g streptomycin/ml and 0.25 mg amphotericin/ml in the absence or presence of different concentrations of OVA (10 or 20 \mu g/ml) or anti-CD3 antibody (1 \mu g/ml). The cells were incubated at 37°C with 5 % CO\textsubscript{2} for 72 h. At 16 h before harvest, 37 kBq [\textsuperscript{3}H]thymidine was added to each well. The cells were harvested and radioactivity was measured with a \beta-counter (Beckman LS 5000 CE; Beckman). The data were expressed as the stimulation index: \( \frac{\text{sample}_{\text{cpm}} - \text{blank}_{\text{cpm}}}{\text{control}_{\text{cpm}} - \text{blank}_{\text{cpm}}} \), where cpm is the counts/min.

Liver cytochrome P450 activity

Livers were homogenized and centrifuged at 12 000 g for 20 min at 4°C. The supernatants were then further ultracentrifuged (105 000 g; XL90, Beckman, Los Angeles, CA, USA) for 1 h and microsomes were isolated. The microsomal suspension was dissolved in buffer for further analysis. Cytochrome P450 content was determined by the dithionite–CO binding difference spectrum at 450 nm (Omura & Sato, 1964).

Ovalbumin-specific proliferative assays

To assay antigen-proliferative response further, spleen cells from OVA-immunized mice were plated in ninety-six-well round-bottomed plates with a concentration of 1 \times 10^6 cells/ml in RPMI 1640 medium supplemented with 20 ml defined serum replacement/l (TCM\textsuperscript{TM}; Celox Co., Hopkins, MN, USA), 4 mM L-glutamine, 25 mM-HEPES, 5 \times 10^{-5} M 2-mercaptoethanol, 100 U penicillin/ml, 100 \mu g streptomycin/ml and 0.25 mg amphotericin/ml in the absence or presence of 5 \mu g lipopolysaccharide/ml and incubated for 24 h. The supernatant fraction was collected and used for determination of PGE\textsubscript{2} and LTB\textsubscript{4} productions. PGE\textsubscript{2} and LTB\textsubscript{4} were determined with an enzyme-linked immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Cytokine assay

Spleen cells, at a concentration of 5 \times 10^6 cells/ml, were cultured with the medium described earlier in the absence or presence of OVA (20 \mu g/ml) for 48 h. Cytokine secretions by single cell suspensions of spleen cells were measured by sandwich-ELISA. Briefly, ninety-six-well flat-bottomed microtiter plates were coated with anti-cytokine antibody diluted in NaHCO\textsubscript{3} buffer, pH 9.6. After overnight incubation at 4°C, plates were washed three times and blocked with bovine serum albumin (30 g/l) at 37°C. After three washes with PBS containing 0.5 ml Tween 20/l, 0.1 ml sample was added for 2 h at 37°C. Plates were then washed with PBS buffer containing 0.5 ml Tween 20/l and biotin-conjugated anti-cytokine antibody diluted in buffer (10 g bovine serum albumin/l PBS) was added and incubated at 37°C for 1 h. After washes, streptavidin-conjugated peroxidase was added for an additional 1 h. The wells were washed, 0.1 ml enzyme substrate of 2,2′-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid solution was added to each well and the plate was left in a dark room for about 30 min. Plates were read in a microplate autoreader at 415 nm. The sensitivity of sandwich-ELISA used in our experiment is 15 pg/ml for IL-4, and 20 pg/ml for IL-5 and interferon (IFN)-γ.

Isolation and stimulation of peritoneal exudate cells

Peritoneal exudate cells were isolated by peritoneal lavage and washed three times with Hanks’ solution before use. Peritoneal exudate cells with a concentration of 1.5 \times 10^6 cells/ml were set up in twenty-four-well plates in RPMI 1640 medium described earlier in the absence or presence of 5 \mu g lipopolysaccharide/ml and incubated for 24 h. The supernatant fraction was collected and used for determination of PGE\textsubscript{2} and LTB\textsubscript{4} productions. PGE\textsubscript{2} and LTB\textsubscript{4} were determined with an enzyme-linked immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Statistical analysis

The significance of difference among four groups was analysed statistically by one-way ANOVA and Duncan’s log multiple range test or Scheffe’s multiple range test of the Statistical Analysis System program system (SAS/STAT version 6; SAS Institute Inc., Cary, NC, USA) throughout the study.

Results

Feed intake and growth

The feed intake and body weights of the mice are shown in Table 3. Although feed intake (g/d) was significantly lower in the 150 g fat/kg groups, the daily energy and protein intakes were only significantly lower in 150-S group. The feed efficiency was significantly lower in the oxidized oil groups. Therefore, gain in body weight and final body weights were the lowest in 150-O group due to its lowest feed efficiency though there was no significant difference in initial body weight before the dietary treatment. The results suggested that the increased content of oxidized oil in the diet did decrease the feed efficiency of mice.

Mice fed oxidized oil (50-O, 150-O) had significantly higher relative liver weight compared with the mice fed fresh oil (Table 4). In contrast, mice of the 150-O group had significantly lower relative heart weight than those of the
Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.

50 g fat/kg 50-S and 50-O groups, and lower relative spleen weights than those of the fresh oil 50-S and 150-S groups. Mice of the 150-O group had a significantly higher hepatic thiobarbituric acid-reactive substance value (nmol/g organ) than that of the other groups (50-S, 0.97 (SE 0.11); 50-O, 0.85 (SE 0.10); 150-S, 1.28 (SE 0.12); 150-O, 1.73 (SE 0.28)). Although the thiobarbituric acid-reactive substance value of spleen was not determined in this study, our previous studies suggest no significant difference between mice fed the fresh oil or oxidized oil diets (Liu & Huang 1995; Lin et al. 1997).

It has been well documented that hepatic cytochrome P450 contents significantly increase in rats fed oxidized oil compared with those of rats fed fresh oil (Huang et al. 1988). Thus, cytochrome P450 is a direct indicator of the dietary effect of oxidized oil. As shown in Table 4, hepatic cytochrome P450 content significantly increased in OVA-immunized BALB/c mice of 150-O group. The effect of oil quantity and quality on cytochrome P450 content was more obvious when data were expressed per g body weight.

### Serum anti-ovalbumin antibodies level

Immunoglobulin (Ig)E anti-OVA antibody tended to be higher in mice of 150-O group than those of the other groups ($P = 0.052$ v. 50-S group, $P = 0.084$ v. 50-O group and $P = 0.057$ v. 150-S group by non-paired Student's $t$ test) (Table 5). Furthermore, the IgG1 anti-OVA antibody level of mice fed 150 g oxidized oil/kg was also significantly higher compared with mice fed 50 or 150 g fresh oil/kg ($P < 0.05$). Both IgE and IgG1 subclasses are affected by type 2 T-helper cell (TH)-related cytokines (Lee et al. 1999). In contrast, TH1-related IgG2a anti-OVA antibody was significantly lower in mice fed high dietary oxidized oil when compared with mice fed fresh oil. All these data suggest that a large amount of dietary oxidized oil could increase TH2-related antigen-specific antibody production and subsequently cause more serious inflammatory response.

### Table 4. Relative tissue weight (g/kg body weight) and liver microsomal cytochrome P450 contents of immunized BALB/c mice

<table>
<thead>
<tr>
<th></th>
<th>50-S Mean</th>
<th>50-S SE</th>
<th>50-O Mean</th>
<th>50-O SE</th>
<th>150-S Mean</th>
<th>150-S SE</th>
<th>150-O Mean</th>
<th>150-O SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>67.0^a</td>
<td>4.1</td>
<td>89.2^a</td>
<td>4.3</td>
<td>56.2^b</td>
<td>3.1</td>
<td>80.8^a</td>
<td>5.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>7.6^a</td>
<td>0.4</td>
<td>6.7^ab</td>
<td>0.6</td>
<td>8.1^a</td>
<td>0.4</td>
<td>5.8^b</td>
<td>0.7</td>
</tr>
<tr>
<td>Hepatic microsomal cytochrome P450 nmol per mg protein</td>
<td>1.2^c</td>
<td>0.1</td>
<td>1.5^bc</td>
<td>0.1</td>
<td>1.6^b</td>
<td>0.1</td>
<td>3.0^a</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>3.5^b</td>
<td>0.2</td>
<td>5.0^c</td>
<td>0.3</td>
<td>5.1^b</td>
<td>0.5</td>
<td>9.4^a</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>6.4^b</td>
<td>0.4</td>
<td>10.5^b</td>
<td>0.8</td>
<td>7.7^b</td>
<td>1.0</td>
<td>20.1^a</td>
<td>3.1</td>
</tr>
<tr>
<td>nmol per g liver</td>
<td>208^c</td>
<td>11</td>
<td>399^b</td>
<td>24</td>
<td>291^c</td>
<td>36</td>
<td>841^a</td>
<td>87</td>
</tr>
</tbody>
</table>

* Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or 150 g oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.
Table 5. Immunological variables of ovalbumin-immunized BALB/c mice fed on 50 g or 150 g fresh oil or oxidized oil/kg diet*  
(Mean values with their standard errors for eight mice per dietary group)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
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<th>SE</th>
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<tbody>
<tr>
<td></td>
<td>50-S</td>
<td>50-O</td>
<td>150-S</td>
<td>150-O</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>OVA-specific immunoglobulin (ELISA units)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IgG1</td>
<td>2.1±1</td>
<td>0.3</td>
<td>2.5³ab</td>
<td>0.4</td>
<td>2.1³b</td>
<td>0.2</td>
<td>3.1³a</td>
<td>0.4</td>
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<tr>
<td>IgG2a</td>
<td>1.0³a</td>
<td>0.1</td>
<td>0.9³b</td>
<td>0.1</td>
<td>1.1³a</td>
<td>0.1</td>
<td>0.7³b</td>
<td>0.1</td>
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<tr>
<td>IgE</td>
<td>0.9±2</td>
<td>0.2</td>
<td>0.9±2</td>
<td>0.2</td>
<td>0.9±2</td>
<td>0.2</td>
<td>1.9±2</td>
<td>0.7</td>
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<tr>
<td>Proliferative response (stimulation index)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>OVA (10 µg/ml)</td>
<td>2.0±2</td>
<td>0.7</td>
<td>3.7±1</td>
<td>1.6</td>
<td>3.0±1</td>
<td>1.9</td>
<td>1.1±2</td>
<td>0.2</td>
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<tr>
<td>OVA (20 µg/ml)</td>
<td>2.6±2</td>
<td>0.8</td>
<td>4.2±1</td>
<td>1.5</td>
<td>5.0±2</td>
<td>3.4</td>
<td>1.2±2</td>
<td>0.2</td>
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<tr>
<td>Anti-CD3 Ab (1 µg/ml)</td>
<td>265±3</td>
<td>136-3</td>
<td>175±7</td>
<td>106-1</td>
<td>104±3</td>
<td>35-7</td>
<td>48±9</td>
<td>19-5</td>
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<tr>
<td>Cytokine levels (ratio)</td>
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<td></td>
</tr>
<tr>
<td>IL-4:IFN-γ</td>
<td>9.1³b</td>
<td>0.3</td>
<td>12³a</td>
<td>0.5</td>
<td>10.5³b</td>
<td>0.7</td>
<td>9.1³b</td>
<td>0.5</td>
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<tr>
<td>IL-5:IFN-γ</td>
<td>0.5³a</td>
<td>0.2</td>
<td>0.5³a</td>
<td>0.1</td>
<td>1.7³b</td>
<td>0.5</td>
<td>1.4³b</td>
<td>0.2</td>
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<tr>
<td>Arachidonic acid metabolites (pg/1x10⁶ cells)</td>
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<td></td>
<td></td>
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<tr>
<td>PGE₂</td>
<td>0.5³b</td>
<td>0.1</td>
<td>1.7³b</td>
<td>0.5</td>
<td>2.9³b</td>
<td>0.9</td>
<td>3.6³a</td>
<td>2.0</td>
</tr>
<tr>
<td>LTΒ₂</td>
<td>49³3</td>
<td>21-9</td>
<td>77³3</td>
<td>40-6</td>
<td>143³b</td>
<td>32-7</td>
<td>392³6</td>
<td>83-9</td>
</tr>
</tbody>
</table>

OVA, ovalbumin; Ig, immunoglobulin; Ab, antibody; IL, interleukin; IFN, interferon; PG, prostaglandin; LT, leukotriene.

*a,b,c,d Mean values within a row with unlike superscript letters were significantly different (P < 0.05; one way ANOVA and Scheffe’s multiple range test).

* Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or 150 g oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.

† Stimulation index = (samplecpm – blankcpm)/(controlcpm – blankcpm), where cpm is the counts per minute.

Discussion

Increasing prevalence of certain allergic diseases such as allergic rhinitis and bronchial asthma has been documented (Aberg et al. 1995). Among the environmental factors, air pollution and dietary habit change have been suggested to play the critical role (Barnes, 1994; Kimber, 1998). Changes in the amount and quality of dietary fat might have an important impact on immunological changes of these atopic diseases. Furthermore, fried food becomes an important fat source in the present-day industrialized dietary pattern, although the relative contribution is difficult to assess. Total energy contributed by dietary fat has been reported to have increased from 30 % during the 1910s to 36 % during the 1980s in the US diet (Committee on Diet and Health Food and Nutrition Board, 1989). The 150 g fresh oil or oxidized oil/kg diet used in the present study provides 31 % total energy from fat.

It has been well documented that diet plays a role in asthma and immune function (Delafuente, 1991; Greene, 1999; Fogarty & Britton, 2000). The data presented here are from one of the few studies to investigate the effect of oxidized oil on immunological changes in a murine model of asthma. Lower relative spleen weight was noted in OVA-immunized BALB/c mice fed on a diet containing 150 g oxidized oil/kg. In addition, lower mitogen-stimulated proliferative response of spleen cell was also noted in mice fed a high amount of oxidized oil, which has also been reported previously (Lin et al. 1997). These data suggested that high amount of oxidized oil intake results in impairment of spleen cells proliferative ability. It will be interesting to study the role of oxidative stress and related enzyme activity in the activation of immune cells further. In addition, it has been documented that TH₁ and TH₂ cells respond differently to distinct population of antigen-presenting cells (Weaver et al. 1988; Gajewski et al. 1991). The effect of antigen-presenting cells and accessory molecules on TH cells’ functions are more pronounced on proliferative response rather than cytokine production ability. This may be the reason for the discrepancy between low proliferative response and active cytokine production in mice in the 150-O group. The data also showed higher IL-5:IFN-γ ratio of cytokine profile produced by T cells of mice fed high amount of dietary fat. Cytokines such as IL-5 derived from TH₂ cells were found to induce eosinophilia, which is critical in the late stage of inflammation of asthma (Marom et al. 1982). In addition, the IL-4:IFN-γ ratio was higher in mice fed 50 g dietary oxidized oil/kg compared with that of the 50-S group. Furthermore, OVA-specific...
IgE and IgG_1 antibody production was not suppressed by dietary oxidized oil. Allergen-specific IgE and mast cells are the effector molecules and cells in triggering inflammatory responses in allergic diseases; however, the central theme of the pathogenic mechanisms involved in allergic diseases is the role of allergen-specific T cells and related cytokines (Ishizaka, 1989; Chretien et al. 1990; Romagnanai, 1990). The result of the present study demonstrated increased TH2-related antigen-specific IgE and IgG_1 antibody in mice fed high dietary oxidized oil. Since the IL-4:IFN-γ ratio was not increased in mice fed high dietary oxidized oil, the higher level of antigen-specific IgE might have resulted from the adjuvant effect of very high levels of PGE_2 and LTB_4 that have been suggested to enhance IgE production (Dugas et al. 1990; Yamaoka et al. 1994). All these data together suggested that both quantity and quality of dietary fat affected the production of antigen-specific IgE and IgG_1 and inflammation-related cytokines.

Inflammatory mediators such as histamine, LT, PG, platelet-activating factor and chemokines have been documented to play a critical role in inflammation of late stage of asthma (Abraham et al. 1983; Russi et al. 1984). Among them, PG, LT and platelet-activating factor are the major metabolites derived from lipids (O’Byrne & Manning, 1992). The present data demonstrated higher PGE_2 and LTB_4 levels in mice fed 150 g dietary oxidized oil/kg compared with those of the other groups. LTB_4, a potent chemotactic agent for the neutrophil, is important in evoking more serious inflammation during the late stage of asthma (Arm et al. 1988). The data also showed that PGE_2 and PGE_1 could increase intracellular cAMP level and negatively regulated TH1 development (Santoli & Zurier, 1989; Betz & Fox, 1991; Gold et al. 1994). Increased PGE_2 and LTB_4 levels might not only aggravate the inflammatory process, but also increase antigen-specific IgE production. Increased intake of dietary oxidized oil could result in increased prostaglandin production and subsequent higher TH2 activity. Higher cytochrome P450 content was noted in mice fed dietary oxidized oil, which is similar to the previous report (Huang et al. 1988). Although microsomal cytochrome P450 activity is more important for PG metabolism, certain enzymes such as PGH synthase implicated in the metabolism of arachidonic acid metabolism may also increase in mice fed oxidized oil (Raz et al. 1989). More studies are needed, however, as dietary oxidized oil may induce higher enzyme activity involved in the pathway of arachidonic acid metabolism and subsequently enhance the production of inflammatory mediators.

It is possible that unidentified fatty acids contained in the oxidized oil (442 g/kg) might play a critical role in the detrimental effect of allergic response. More studies on the identification of the possible components are needed. The formation of volatile and non-volatile products in dietary oxidized oil has been reported (Chang et al. 1978). The major compounds formed during the frying process and left in oxidized oil are non-volatile. These non-volatile compounds can only be identified as non-urea-adduct-forming esters. The polymers formed during deep-fat frying were essentially dimers and trimers, which made the identification of these components relatively difficult. However, it would be very informative if the active substance in oxidized oil responsible for the changes of inflammatory response could be identified in the future.

These present data demonstrated that increasing content of dietary oxidized oil in modern food could increase both IgE, the IL-4:IFN-γ or IL-5:IFN-γ ratio and inflammatory mediators such as PGE_2 and LTB_4, which are all hazardous for the disease severity of asthma.

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References


Oxidized oil and allergic disease

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