



Dietary perilla oil lowers serum lipids and ovalbumin-specific IgG1, but increases total IgE levels in ovalbumin-challenged mice

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ABSTRACT

Our previous studies indicated that α -linolenic acid (ALA)-rich perilla oil might alleviate bronchoalveolar inflammation. However, it failed to modulate the Th1/Th2 balance toward the Th1 pole during Th2-skewed allergic airway inflammation in mice. This study attempts to further investigate the effects of dietary perilla oil on serum lipids and immunoglobulin profiles using an ovalbumin (OVA)-challenged mouse model. The inbred female BALB/c mice were randomly divided into four groups and fed different AIN-76 feeds containing 5% corn oil (rich in linoleic acid, 18:2n-6 polyunsaturated fatty acids (PUFA), as a control diet), 5% perilla oil (rich in α -linolenic acid, 18:3n-3 PUFA) or 5% compound oil containing 50% corn oil and 50% perilla oil, respectively, for 35 consecutive days *ad libitum*. Experimental mice were sensitized by an intraperitoneal injection of alum-precipitated antigen containing ovalbumin on 7, 14 and 21 days after supply of the specified experimental diets. One week later, the mice were then challenged by aerosolized OVA. The results showed that dietary perilla oil administration significantly ($P < 0.05$) decreased the relative liver tissue weight (RTW) and serum lipid levels including triglycerides, total cholesterol, HDL- and LDL-cholesterol. However, the HDL/LDL ratio was also significantly lowered by dietary perilla oil. Dietary perilla oil markedly decreased serum OVA-specific IgG1 level and total IgA antibodies (Th2 antibodies). Unfortunately, it also increased non-specific serum IgE (Th2 antibody) levels. The results suggest that dietary perilla oil might have a moderately beneficial effect on asthmatic allergy via lowering serum lipids and OVA-specific IgG1, as well as total IgA levels. However, it failed to obviously modulate Th1/Th2 antibody levels via isotype switching of B cells from Th2 antibody to Th1 antibody.

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1. Introduction

Changes in dietary lipid pattern, such as imbalance in the n-6/n-3 polyunsaturated fatty acids (PUFAs), may be promoters of many chronic diseases including atherosclerosis, essential hypertension, obesity, diabetes and many cancers due to the increased production of pro-inflammatory cytokines and eicosanoids *in vivo* (Simopoulos, 1991, 2002a). Different kinds of dietary fat with varying fatty acid compositions have been judged to influence plasma and tissue lipid appearance (Yamamoto et al., 1987; Surette et al., 2004). The positive or negative impacts on serum lipid profiles by dietary fats are associated with human health (Wahrburg, 2004; Connor, 2000). The effects of n-6/n-3 fatty acid ratios in dietary oil on health have been much discussed.

Long-chain n-3 (PUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from oily fish

and fish oil are reported to have beneficial biological effects in improving human health (Simopoulos, 2002b; Sijben and Calder, 2007). Dietary n-3 PUFAs reduces the risk for coronary heart disease by lowering the heart rate and blood pressure, decreasing platelet aggregation and lowering triglyceride levels (Harris et al., 2008). Both EPA and DHA can attenuate T-cell-mediated inflammation (Switzer et al., 2004), decrease the production of arachidonic acid-derived mediators and pro-inflammatory cytokines, and modulate adhesion molecule expression (Calder, 2005).

Perilla frutescens is an edible plant frequently used as one of the most popular garnishes and food colorants in some Asian countries such as China and Japan. Perilla leaf extract has been reported to possess several anti-inflammatory effects, such as inhibition of tumor necrosis factor- α production in male ICR mice (Ueda et al., 2002) and inhibition of seasonal allergic rhinoconjunctivitis in humans (Takano et al., 2004). Perilla seeds are widely used to treat pulmonary diseases in traditional Chinese medicine. Perilla oil from *P. frutescens* seeds is rich in α -linolenic acid (ALNA, 18:3n-3). ALNA is a plant-derived n-3 fatty acid, which exists in green leafy vegetables, as well as some seed and vegetable oils, is a

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precursor of EPA and DHA (Burdge and Calder, 2005). ALNA-rich perilla oil exerts a positive effect on preventing atherosclerosis and chemically induced cancer, and improves immune and mental function in animal studies (Kurowska et al., 2003). Kim and Choi (2001) indicated that feeding perilla oil diets decreased postprandial plasma lipids in male Sprague-Dawley rats. Dietary perilla oil also exhibits a similar physiological activity to fish oil in modulating hepatic fatty acid oxidation (Ide et al., 2000; Kim et al., 2004). We have also shown that dietary perilla oil inhibits pro-inflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice (Chang et al., 2008). However, more evidence concerning the physiological function of dietary perilla oil should be identified, especially under the stress of different diseases such as asthma.

A clinical study has indicated that hypercholesterolemia is a potential risk factor for asthma (Al-Shawwa et al., 2006). Yeh and Huang (2004) reported that allergic pulmonary inflammation is significantly correlated with serum cholesterol levels in male C57BL/6 mice sensitized with ovalbumin. Surprisingly, simvastatin, widely used in clinical practice as cholesterol-lowering agents, is effective in reducing BAL total cellularity and eosinophilia in a murine model of allergic asthma (McKay et al., 2004). Recent human and animal studies suggest that hypercholesterolemia is associated with high levels of inflammation-sensitive plasma proteins (Engström et al., 2002), as well as increased expression of pro-inflammatory genes, cellular adhesion molecules, and pro-inflammatory cytokines (Stokes et al., 2002; Scalia et al., 1998). Thus, serum lipid profiles may be related to immunological responses during inflammation.

To understand the effects of dietary perilla oil on the allergic asthma, we have established an asthmatic mouse model to evaluate the anti-inflammatory and immuno-modulatory potential of ALNA-rich perilla oil (Chang et al., 2008; Lin et al., 2006a; Nagai et al., 2004). The female BALB/c mice were divided into four groups and fed different AIN-76 feeds containing 5% corn oil (rich in linoleic acid, 18:2n-6 polyunsaturated fatty acids (PUFA), as a control diet), 5% perilla oil (rich in α -linolenic acid, 18:3n-3 PUFA) or 5% compound oil containing 50% corn oil and 50% perilla oil, respectively, for 35 consecutive days *ad libitum*. Experimental mice were sensitized by an intraperitoneal injection (i.p.) of alum-precipitated antigen containing ovalbumin on 7, 14 and 21 days after supply of the specified experimental diets. One week later, the mice were then challenged by aerosolized OVA. We found that perilla oil administration might alleviate bronchoalveolar inflammation by decreasing the secretion of pro-inflammatory cytokines and T-helper type 1 (Th1) cytokines into the local lung and airway tissues compared to corn oil (rich in linoleic acid, LA, 18:2n-6). However, asthma is recognized as a Th2-skewed allergic disease (Kay, 2003). Th2-type cells are important in humoral immunity and defend against extracellular pathogens (Opal and DePalo, 2000) via eosinophilic infiltration, as well as IgE and IgG1 production *in vivo* (Morokata et al., 1999). In contrast to the Th2 cells, Th1 cells promote cell-mediated immunity to destroy intracellular pathogens (Opal and DePalo, 2000), to inhibit eosinophilic infiltration, IgE and IgG1 production, but strengthen IgG2a production *in vivo* (Morokata et al., 1999). Th1-skewed immune responses are generally pro-inflammatory and may result in autoimmune and chronic inflammatory diseases, while Th2-skewed immune responses may cause asthma and other allergies (Kidd, 2003). Although the Th1/Th2 paradigm is well known, there is little discussion on the regulation of immunoglobulin and serum lipid profiles by ALNA-rich perilla oil in an allergic airway inflammation murine model. This study investigates the effects of dietary perilla oil on serum lipids and antibody levels using an ovalbumin (OVA)-sensitized and challenged allergic airway inflammation murine model.

2. Methods

2.1. Materials, experimental animals and feeds

The feed formula was same as that described by Chang et al. (2008). The experimental feed was prepared according to the recommendation of the American Institute of Nutrition AIN-76 (AIN, 1977) that satisfies the nutritional requirement for mouse growth, and varied only in lipid composition. We just replaced the oil component (5%) in AIN-76 feed with the selected experimental oils (5%). The diets are nutritionally balanced. The basic composition of each feed, expressed in g/100 g, contained 40 g sucrose, 25 g corn starch, 20 g casein, 5 g fiber, 3.5 g mineral mixture, 1 g vitamin mixture, 0.3 g DL-methionine, 0.2 g choline bitartrate and 5 g lipid. The components of each feed were thoroughly mixed and stored at -20°C . There were three kinds of lipid used in the experiment. Perilla oil (PER) rich in α -linolenic acid (>60.5%) was purchased from MasterAsia Marketing Co. (Taipei, Taiwan, ROC) as the source of n-3 fatty acid. In addition, there are 14.7% linoleic acid, 16.1% oleic acid, 2.0% stearic acid and 6.2% palmitic acid in perilla oil according to the manufacturer's profiles. Corn oil (COR), rich in linoleic acid but poor in α -linolenic acid (<2%), was purchased from Chang Chi Foodstuff Factory Co. (Taichung, Taiwan, ROC) as a control and the source of n-6 fatty acid. The fatty acid composition in corn oil consists of 49.3% linoleic acid, 28.5% oleic acid, 2.1% stearic acid and 12.9% palmitic acid according to the data of Rafalowski et al. (2008). The ratios of n-6/n-3 fatty acids are 0.27 in perilla oil and 61.6 in corn oil, respectively. Compound oil (COR-PER) was a mixture containing 50% corn oil and 50% perilla oil.

The animal use protocol listed below has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), National Chung Hsing University, Taiwan, ROC. The female BALB/c ByJNarl mice (6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained in the Department of Food Science and Biotechnology at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The animal room was kept on a 12-h light and 12-h dark cycle. Constant temperature ($25 \pm 2^{\circ}\text{C}$) and humidity were maintained. The mice were housed and kept on a chow diet (laboratory standard diet) to acclimatize for 2 weeks before feeding the experimental diet. After this equilibrium period, mice were randomly divided into four groups ($n = 12$) varied by oil source and sensitized treatments: non-sensitized control (PBS/COR), dietary control (OVA/COR), OVA/COR-PER and OVA/PER. The initial average body weight of each group showed no significant differences among groups. Each group was fed with the specified experimental diet for 35 consecutive days *ad libitum*. Mice food intake and body weight were measured twice a week during the study period.

2.2. OVA-sensitized and -challenged allergic inflammation murine model

The inflammation murine model was used in our previous study (Chang et al., 2008). The mice (8 weeks old) were sensitized and challenged to induce allergic airway inflammation. The mouse allergic airway inflammation model was manipulated as described by Lin et al. (2006a) and slightly modified at aerosolized-OVA concentration and inhalation time to enhance the induction of airway inflammation. In brief, mice were sensitized by an intraperitoneal injection (i.p.) of 0.2 ml alum-precipitated antigen containing 8 μg of ovalbumin (OVA, albumin chicken egg grade III, Sigma, MO, USA) and 2 mg $\text{Al}(\text{OH})_3$ to induce primary immunity after supply of the specified experimental diets for one week. Two booster injections of this alum-OVA mixture were given 7 and 14 days later, respectively. Non-sensitized control mice received alum-phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , pH 7.2–7.4, 0.2 μm filtered) only. One week later, the mice were then challenged by aerosolized OVA at a concentration of 5 mg OVA per milliliter PBS for 60 min, twice at 3 day intervals and repeated twice in 24 h. The aerosolized OVA were produced using an ultrasonic nebulizer (sw918, Shinmed). Non-sensitized control mice received only PBS. Two days later, the animals were anesthetized with diethyl ether, exsanguinated by retro-orbital venous plexus puncture and immediately euthanized by CO_2 inhalation. The serum was collected and assayed for antibody and lipid contents. The organs were collected and weighted.

2.3. Assay of total triglycerides, cholesterol, HDL- and LDL-cholesterol

The lipid levels in experimental mouse serum were determined using Randox assay kits: triglycerides (TG) assay kit, total cholesterol (TC) assay kit and high density lipoprotein (HDL)-cholesterol assay kit. Serum samples were appropriately diluted. The lipid levels were assayed according to the manufacturer's instructions. The concentration of low density lipoprotein (LDL)-cholesterol was calculated using the following formula: $\text{LDL-cholesterol (mg/dl)} = \text{total cholesterol} - (\text{TG}/5) - \text{HDL}$.

2.4. OVA-specific IgE, IgG1 and IgG2a assay

An ELISA protocol was used to determine the OVA-specific IgE, IgG1 and IgG2a antibody levels in the serum. Aliquots of 200 μl /well OVA (10 $\mu\text{g}/\text{ml}$ dissolved in 0.1 M NaHCO_3 , pH 8.2) were pipetted into the 96-microwell plate (Nunc), respectively. Plates were incubated overnight at 4°C . The plates were then carefully

washed with wash buffer (0.05% Tween20 in PBS buffer) three times. Serum samples were appropriately diluted before they were respectively added to the plate wells. Aliquots of 100 μ l of diluted serum samples were respectively added to the wells. The OVA-specific levels were then determined using the biotin-conjugated rat anti-mouse IgE monoclonal antibody, biotin-conjugated rat anti-mouse IgG1 monoclonal antibody and biotin-conjugated rat anti-mouse IgG2a monoclonal antibody (BD Biosciences). The manipulation was according to the manufacturer's instructions for use. The plates were finally read on a plate reader (ASYS Hitech GmbH, Austria) at 450 nm. Absorbency (A) was measured at 450 nm with the ELISA reader and optical densities were converted into arbitrary ELISA units (EU) (Schmid-Grendelmeier et al., 2005). The ELISA unit = $(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{positive}} - A_{\text{blank}})$.

2.5. Non-specific antibody quantification by ELISA

Serum antibody levels of IgA, IgE, IgG, and IgM were analyzed using the mouse IgA, IgE, IgG, and IgM ELISA quantitation kits (Bethyl Laboratories, Inc., TX, USA). Serum samples were appropriately diluted. Briefly, IgA, IgE, IgG, and IgM levels in serum were measured using a sandwich enzyme-linked immuno-absorbent assay (ELISA). Micro-titre plate wells (flat bottom; high binding; Costar, Corning, NY, USA) were respectively coated with aliquots of 100 μ l of goat anti-mouse IgA-, IgE-, IgG-, and IgM-affinity purified antibody at a concentration of 10 μ g/ml carbonate buffer (0.05 M sodium carbonate, pH 9.6) for 60 min at room temperature. After the plate wells were washed twice using wash buffer (50 mM Tris, pH 8.0, 0.1 M NaCl, 0.05% Tween 20), aliquots of 200 μ l blocking solution (1% BSA, 50 mM Tris, pH 8.0, 0.15 M NaCl) were post-coated to each well for 30 min. After the plate wells were washed twice using wash buffer (50 mM Tris, pH 8.0, 0.1 M NaCl, 0.05% Tween 20), aliquots of 100 μ l purified mouse IgA, IgE, IgG, and IgM standards which serially diluted in diluent (1% BSA, 50 mM Tris, pH 8.0, 0.15 M NaCl, 0.05% Tween 20) and pre-diluted serum samples were added to the wells. The plate was then incubated for 60 min at room temperature. After the plate wells were washed twice using wash buffer, aliquots of 100 μ l horseradish peroxidase-conjugated goat anti-mouse IgA, IgE, IgG, and IgM antibody were respectively added to wells for 60 min. After washing three times again, the plate wells were developed by adding aliquots of 100 μ l substrate solution containing 3,3',5,5'-tetramethyl benzidine (TMB) and incubated for 5–30 min (Lin et al., 2006b). The reaction was stopped by adding an aliquot of 100 μ l of 2 M sulfuric acid per well and the absorbance at 450 nm was measured with an ELISA reader (ASYS Hitech GmbH, Austria).

2.6. Statistical analysis

Data are expressed as mean $\pm$ SD. Data are analyzed using analysis of variance (ANOVA) followed by Duncan's test and unpaired Student's *t*-test. Differences between the dietary control and non-sensitized control or among OVA-sensitized groups were considered statistically significant if $P < 0.05$. Linear relations were determined as Pearson's correlation coefficients (*r*), and a value of $P < 0.05$ indicated statistical significance. Statistical tests were performed using SPSS version 12.0.

3. Results

3.1. Intake and growth

The body weight and experimental procedure during the experimental period are given in Fig. 1. The mean body weight of all mice slightly increased as experimental period extended, however there were no significant differences among groups. The feed efficiency and body weight changes of experimental mice are shown in Table 1. There were no significant differences in feed intake, feed efficiency and body weights among the four experimental groups. However, administration with compound oil (OVA/COR-PER group) markedly ($P < 0.05$) increased gain in body weight compared to that of dietary perilla oil (OVA/PER group). OVA-sensitization and -challenge resulted in slight, but not significant, increases in absolute and relative liver weights (PBS/COR vs. OVA/COR, Table 2). However, dietary perilla oil, whether perilla oil alone (OVA/COR) or compound oil containing perilla oil and corn oil (OVA/COR-PER), significantly decreased the relative liver weights (decrease from 6.36 ± 0.26 to $5.65 \pm 0.24\%$ (–11.2%)) of experimental mice (Table 2).

3.2. Serum lipid levels

The serum lipid levels of experimental mice are shown in Table 3. The OVA-sensitization and challenge did not significantly influence serum lipid levels (OVA/COR vs. PBS/COR, Table 3), although the treatments slightly increased in serum triglycerides, cholesterol, HDL-, and LDL-cholesterol levels. However, dietary perilla oil, whether perilla oil alone (OVA/COR) or compound oil containing perilla oil and corn oil (OVA/COR-PER), markedly decreased serum lipid levels. Serum triglycerides decreased from 69.3 ± 36.5 to 28.0 ± 11.0 mg/dl (–59.6%), cholesterol decreased from 175 ± 36 to 115 ± 17 mg/dl (–34.3%), HDL-cholesterol decreased from 44.8 ± 8.7 to 23.2 ± 4.3 mg/dl (–48.2%) and LDL-cholesterol decreased from 116 ± 35 to 83 ± 14 mg/dl (–28.4%). The ratios of HDL/cholesterol and HDL/LDL decreased from 0.26 ± 0.06 to 0.19 ± 0.02 (–26.9%) and 0.37 ± 0.09 to 0.26 ± 0.04 (–29.7%), respectively.

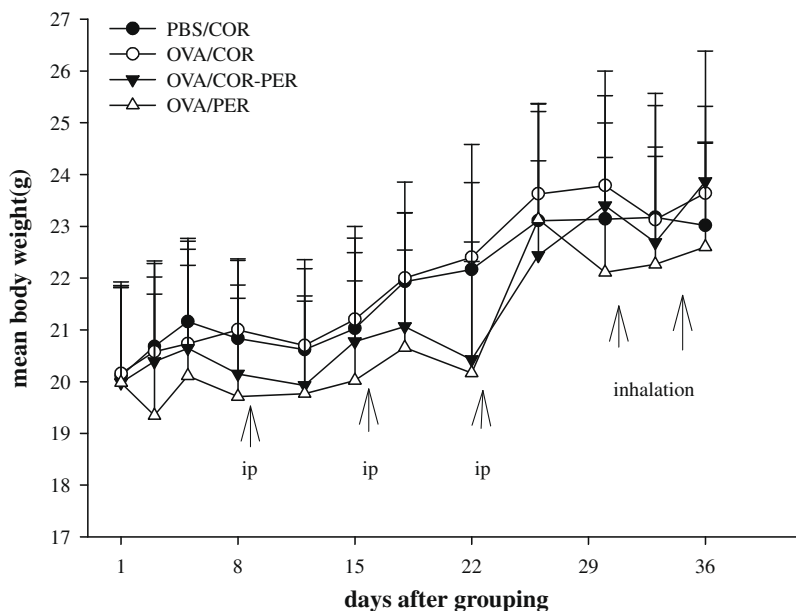


Fig. 1. Effects of different dietary oil on body weights of BALB/c mice in an OVA-sensitized and -challenged asthmatic murine model. Mice were sensitized with OVA/alum at days 7, 14 and 21. The sensitized mice were then challenged twice with aerosolized OVA for 60 min twice a day at days 30 and 33. All experimental mice were sacrificed at day 35 and their sera were collected and analyzed. i.p.: Mice were intraperitoneally injected with OVA or PBS. Inhalation: mice inhaled aerosolized OVA or PBS.

Table 1Effects of different dietary oils on body weight changes and feed efficiency^a of OVA-sensitized and -challenged BALB/c mice.

Items	Groups			
	OVA/COR	OVA/COR-PER	OVA/PER	PBS/COR
Initial body weight (g) ^b	20.46 ± 1.53A	20.03 ± 2.00A	19.98 ± 1.95A	20.11 ± 1.70A
Final body weight (g) ^b	23.64 ± 0.99A	23.86 ± 2.52A	22.60 ± 2.01A	23.02 ± 2.30A
Gain in body weight (g) ^b	3.18 ± 0.69AB	3.83 ± 0.82B	2.62 ± 1.01A	2.91 ± 0.83A
Feed intake (g/d) ^b	3.60 ± 0.44A	3.54 ± 0.23A	3.44 ± 0.31A	3.57 ± 0.35A
Feed efficiency (%) ^b	2.50 ± 0.52A	2.91 ± 0.15A	2.15 ± 0.45A	2.28 ± 0.43A

^a Data are represented as mean ± SD (*n* = 8–12) and analyzed using analysis of variance (ANOVA) followed by Duncan's test.^b Values within same row not sharing common capital letters are significantly different (*P* < 0.05) from each other.**Table 2**Effects of different dietary oils on absolute and relative liver weight^a of OVA-sensitized and -challenged BALB/c mice.

Groups	Absolute liver weight (g) ^b	Relative liver weight (%) ^b
OVA/COR	1.39 ± 0.07A	6.36 ± 0.26C
OVA/COR-PER	1.34 ± 0.18A	6.03 ± 0.23B
OVA/PER	1.25 ± 0.19A	5.65 ± 0.24A
PBS/COR	1.34 ± 0.15 ^c	6.29 ± 0.51 ^c

^a Data are represented as mean ± SD (*n* = 7–12) and analyzed using analysis of variance (ANOVA) followed by Duncan's test.^b Values within same column not sharing common capital letters are significantly different (*P* < 0.05) from each other.^c There are no significant differences between OVA/COR and PBS/COR groups within same column analyzed by unpaired Student's *t*-test.

3.3. Serum non-specific (total) and OVA-specific antibody levels

To understand the effects of perilla oil administration on serum antibody levels in experimental mice, OVA-specific IgE, IgG1, and IgG2a (Table 4), as well as total IgA, IgG, IgM, and IgE levels (Table 5) were determined. The results indicated that the OVA-sensitization and -challenge markedly (*P* < 0.05) increased serum OVA-specific IgE, IgG1, and IgG2a as well as total IgE levels, but not IgA, IgG and IgM (Tables 4 and 5). Administration with perilla oil, whether perilla oil alone (OVA/PER) or compound oil containing perilla oil and corn oil (OVA/COR-PER), markedly (*P* < 0.05) decreased serum IgG1 and total IgA levels, but increased total IgE levels in sera. The levels of serum IgG1 and total IgA decreased from 1.28 ± 0.08 to 0.99 ± 0.07 (–22.6%, Table 4) and from 1832 ± 297 to 1515 ± 260 µg/ml (–17.3%, Table 5) respectively, total IgE levels increased from 5.38 ± 1.85 to 7.57 ± 1.50 µg/ml (+28.9%, Table 5). However, perilla oil administration did not significantly affect OVA-specific IgE, and IgG2a, as well as total IgG, and IgM levels.

3.4. Relationship among serum lipid content, OVA-specific or non-specific antibody levels of OVA-sensitized and -challenged BALB/c mice

To further understand the relationship among serum lipid content, OVA-specific or non-specific antibody levels in OVA-sensi-

Table 4Effects of different dietary oil on serum OVA-specific antibody titers^a of OVA-sensitized and -challenged BALB/c mice.

Groups	Antibody titers ^b (ELISA unit ^c)		
	IgE ^d	IgG1 ^d	IgG2a ^d
OVA/COR	1.37 ± 0.59A	1.28 ± 0.08B	0.84 ± 0.35A
OVA/COR-PER	1.84 ± 0.64A	1.03 ± 0.08A	0.70 ± 0.41A
OVA/PER	1.95 ± 0.90A	0.99 ± 0.07A	0.79 ± 0.38A
PBS/COR	0.00 ± .01 ^c	0.03 ± 0.03 ^c	0.03 ± 0.02 ^c

^a Data are shown as mean ± SD (*n* = 8–12).^b Values within same column not sharing common capital letters are significantly different (*P* < 0.05) from each other analyzed by analysis of variance (ANOVA) followed by Duncan's test.^c Asterisk (*) means significantly (*P* < 0.05) different between OVA/COR and PBS/COR groups within same column analyzed by unpaired Student's *t*-test.^d Sera were respectively diluted to 1/10 for IgE, 1/250 for IgG2a and 1/25,000 for IgG1 determinations.^e ELISA unit = (*A*_{sample} – *A*_{blank})/(*A*_{positive} – *A*_{blank}).

tized and -challenged BALB/c mice, the correlations between serum lipid and antibody levels were determined using Pearson's correlation coefficients (*r*). The results showed a significantly negative correlation between OVA-specific IgE and TC levels in overall OVA-challenged groups (Table 6). However, there were significantly positive correlations among OVA-specific IgG1 levels and TG, TC, HDL or LDL levels in total OVA-challenged groups. There was no significant correlation among the OVA-specific IgG2a level and serum lipid levels. Further analysis showed that serum non-specific IgE levels were conversely related to TG and HDL levels, as well as the HDL/LDL ratio. However, non-specific IgA levels were positively related to TC, HDL, and LDL levels in all experimental mice (Table 6). There was a significantly negative correlation between non-specific IgM levels and the HDL/LDL ratio in all experimental mice.

4. Discussion

Many reports have indicated that n-3 PUFAs demonstrated immune effects on inflammatory mediators and pro-inflammatory

Table 3Effects of different dietary oils on serum lipid levels^a of OVA-sensitized and -challenged BALB/c mice.

Items	Groups			
	OVA/COR	OVA/COR-PER	OVA/PER	PBS/COR
Triglycerides (mg/dl) ^b	69.3 ± 36.5B	41.0 ± 10.4A	28.0 ± 11.0A	55.1 ± 20.8 ^c
Cholesterol (mg/dl) ^b	175 ± 36B	137 ± 23A	115 ± 17A	158 ± 24 ^c
HDL-cholesterol (mg/dl) ^b	44.8 ± 8.7B	28.2 ± 7.7A	23.2 ± 4.3A	42.8 ± 5.2 ^c
LDL-cholesterol (mg/dl) ^b	116 ± 35B	100 ± 19AB	83 ± 14A	100 ± 25 ^c
HDL/cholesterol (mg/mg) ^b	0.26 ± 0.06B	0.19 ± 0.03A	0.19 ± 0.02A	0.30 ± 0.07 ^c
HDL/LDL (mg/mg) ^b	0.37 ± 0.09B	0.25 ± 0.04A	0.26 ± 0.04A	0.38 ± 0.05 ^c

^a Data are shown as mean ± SD (*n* = 8–12).^b Values within same row not sharing common capital letters are significantly different (*P* < 0.05) from each other analyzed by analysis of variance (ANOVA) followed by Duncan's test.^c There are no significant differences within same row between OVA/COR and PBS/COR groups analyzed by unpaired Student's *t*-test.

Table 5

Effects of different dietary oil on non-specific immunoglobulin levels^a in sera from OVA-sensitized and -challenged BALB/c mice.

Groups	Antibody levels ^b (μg/ml)			
	IgA ^d	IgG ^d	IgM ^d	IgE ^d
OVA/COR	1832 ± 297B	388 ± 193A	1392 ± 204A	5.38 ± 1.85A
OVA/COR-PER	1406 ± 373A	433 ± 228A	1465 ± 220A	5.78 ± 2.23AB
OVA/PER	1515 ± 260A	483 ± 225A	1539 ± 245A	7.57 ± 1.50B
PBS/COR	1699 ± 429	462 ± 237	1371 ± 107	0.35 ± 0.21 ^c

^a Data are represented as mean ± SD (n=8–12).

^b Values within same column not sharing common capital letters are significantly different ($P < 0.05$) from each other analyzed by analysis of variance (ANOVA) followed by Duncan's test.

^c Asterisk (*) means significantly ($P < 0.05$) different between OVA/COR and PBS/COR groups within same column analyzed by unpaired Student's *t*-test.

^d Sera were respectively diluted to 1/100 for IgE, 1/5000 for IgA and IgM, 1/10,000 for IgG determinations.

Table 6

The correlation analyses between serum antibody levels and serum lipid levels in total experimental groups.

	TG	TC	HDL	LDL	HDL/LDL
<i>OVA-specific antibodies vs. serum lipid levels (n = 25)</i>					
IgE	$r = -0.173$ $P = 0.408$	$r = -0.411^*$ $P = 0.041$	$r = -0.340$ $P = 0.097$	$r = -0.367$ $P = 0.071$	$r = 0.004$ $P = 0.985$
IgG1	$r = 0.616^*$ $P = 0.001$	$r = 0.720^*$ $P = 0.000$	$r = 0.726^*$ $P = 0.000$	$r = 0.522^*$ $P = 0.007$	$r = 0.369$ $P = 0.070$
IgG2a	$r = -0.127$ $P = 0.544$	$r = 0.041$ $P = 0.846$	$r = -0.077$ $P = 0.713$	$r = 0.115$ $P = 0.586$	$r = -0.145$ $P = 0.488$
<i>Non-specific antibodies vs. serum lipid levels (n = 37)</i>					
IgE	$r = -0.387^*$ $P = 0.018$	$r = -0.305$ $P = 0.066$	$r = -0.572^*$ $P = 0.000$	$r = -0.031$ $P = 0.855$	$r = -0.462^*$ $P = 0.004$
IgA	$r = 0.086$ $P = 0.613$	$r = 0.494^*$ $P = 0.002$	$r = 0.378^*$ $P = 0.021$	$r = 0.428^*$ $P = 0.008$	$r = 0.041$ $P = 0.809$
IgG	$r = -0.053$ $P = 0.757$	$r = 0.200$ $P = 0.235$	$r = -0.019$ $P = 0.911$	$r = 0.274$ $P = 0.101$	$r = -0.237$ $P = 0.158$
IgM	$r = -0.256$ $P = 0.126$	$r = 0.072$ $P = 0.672$	$r = -0.214$ $P = 0.203$	$r = 0.249$ $P = 0.137$	$r = -0.353^*$ $P = 0.032$

* Correlations were considered statistically significant if $P < 0.05$.

cytokines based on human or experimental animal studies (Sijben and Calder, 2007; Switzer et al., 2004). To unravel the effects of dietary perilla oil on serum lipids and antibodies in the allergic asthmatic disease, we used an established allergic asthma animal model (Chang et al., 2008) to evaluate the effects of perilla oil (rich in ALNA, 18:3n-3) or corn oil (rich in LA, 18:2n-6) on serum lipids and antibody productions in this study. It was found that dietary perilla oil (2.5% or 5% in diet) significantly decreased the relative liver weight (Table 2) and serum lipid levels, including triglycerides, cholesterol, HDL-cholesterol, and LDL-cholesterol (Table 3). The results suggest that dietary perilla oil might decrease lipid accumulation in the liver, however it remains to be further clarified. Decreases in lipid accumulation in the liver with serum lipids such as triglycerides, cholesterol, and LDL-cholesterol might be helpful for health. However decreases in HDL-cholesterol, and the ratios of both HDL/cholesterol and HDL/LDL were considered detrimental to human health. Javadi et al. (2007) demonstrated that feeding high levels of ALNA (12% in diet) decreased plasma total cholesterol, HDL-cholesterol and phospholipids in male BALB/cAnNrl mice. Kim and Choi (2001) also found that perilla oil exerted an effect similar to that of fish oil in lowering postprandial plasma triacylglycerol and total cholesterol level in male Sprague-Dawley rats. Our results confirmed the hypolipidemic effects of dietary perilla oil in the allergic asthma mouse model. Hypercholesterolemia is recognized as a potential risk factor for asthma

(Al-Shawwa et al., 2006), therefore the hypolipidemic effects of dietary perilla oil are suggested to reduce the risk for allergic asthma. Leyton et al. (1987) demonstrated that ALNA has a faster β -oxidation rate than LA using labeled fatty acids in female Sprague-Dawley rats. ALNA-rich perilla oil has been reported to stimulate hepatic activity and acyl-CoA oxidase gene expression. This partly accounts for the lower plasma triglyceride levels in rats (Kim and Choi, 2005). Accordingly, we suggest that the hypolipidemic effects of perilla oil might result from the faster ALNA β -oxidation rate and poor lipogenesis in lipid metabolism in this study.

Although the benefits of n-3 PUFA on autoimmune and inflammatory diseases are recognized (Simopoulos, 2002b), their protective effects on asthma are still equivocal (Mickleborough and Rundell, 2005). Our results exhibited that OVA-sensitized and challenged treatment significantly increased serum OVA-specific IgE (Th2 antibody), IgG1 (Th2 antibody) and IgG2a (Th1 antibody), and total (non-specific) IgE antibody levels (Tables 4 and 5). Perilla oil administration significantly decreased serum OVA-specific IgG1 (Th2 antibody) titers in both OVA/COR-PER and OVA/PER groups compared to that of OVA/COR groups (Table 4), suggesting that dietary perilla oil may have an anti-allergic potential against allergic asthmatic diseases in BALB/c mice. Unfortunately, in this study perilla oil administration also increased serum non-specific IgE titers, but decreased non-specific IgA titers (Table 5). IgA may serve as the second line of defense mediating the elimination of pathogens that have breached the mucosal surface (Woof and Kerr, 2004) and be defined as inflammatory antibody through its interaction with Fc α -RI on other immune cells, including macrophages, neutrophils and eosinophils (Otten and van Egmond, 2004). Speculatively, the decrease in serum IgA might be beneficial to the asthmatic allergic inflammation. In contrast, the increase in total IgE titers might activate mast-cells through cross-linking on the mast-cell surface, leading to rapid release of inflammatory mediators. The results suggest that dietary perilla oil might have a biphasic effect on non-specific antibody productions such as IgE and IgA. However, we still suggest that the overall effects of dietary perilla oil on allergic asthmatic diseases are beneficial through decreasing OVA-specific IgG1 titers in sera. A recent study indicated that long-chain n-3 PUFAs dampen excessive inflammation in the inflammatory diseases and statuses through the suppression of eicosanoids, and inhibition of IgE production by B cells (Sijben and Calder, 2007). ICR mice, fed with perilla oil (10% in diet) and immunized with 10μg of egg albumin exhibited significantly lower titers of serum anti-egg albumin IgE antibody than those from safflower oil (rich in 18:2n-6) group (Watanabe et al., 1994). Conversely, Albers et al. (2002) reported that n-3 PUFA diet (rich in EPA and DHA) significantly increased dinitrochlorobenzene(DNCB)-specific IgE levels in DNCB-sensitized BALB/c mice. In this study dietary perilla oil (5% in diet) also slightly raised serum OVA-specific IgE and markedly ($P < 0.05$) increased total serum IgE levels (Tables 4 and 5). We speculate that differences in mouse species, dietary perilla oil level or immunization model may cause slightly divisive results. We believe that the results from this study presenting the effects of (5% in diet) of dietary perilla oil levels on allergic asthmatic diseases are valuable. The effects of dietary perilla oil on antibody isotype switching *in vivo* remain for further study.

Surprisingly, OVA-specific, or non-specific IgE levels were negatively correlated with serum lipids (Table 6). Non-specific IgA levels were positively correlated with serum lipids in this study (Table 6). Non-specific IgM levels were negatively correlated with the ratio of HDL/LDL in experimental mice (Table 6). These results suggest that n-3 and n-6 PUFAs may exert an ability to modulate B cell immunity in concert with their regulation of lipid metabolism. It has been reported that ALNA and its metabolites (EPA or DHA) might alter the lipid rafts in immune cells' plasma membrane and modulate the cellular signaling in immune system (Ma et al.,

2004). A correlation between serum antibody levels and lipid profiles may exist. However, the regulatory effects of dietary fats on antibody titers need to be further clarified.

We have concluded a conclusion about our previous study (Chang et al., 2008) that dietary perilla oil might alleviate inflammation via decreasing the secretion of pro-inflammatory cytokines in bronchoalveolar lavage fluid, but failed to regulate the Th1/Th2 balance toward Th1 pole according to the Th1/Th2 (IFN- γ /IL-4, IFN- γ /IL-5, and IFN- γ /(IL-4+IL-5)) cytokine secretion profiles during the Th2-skewed allergic airway inflammation. In this study we found that dietary perilla oil significantly ($P < 0.05$) increased non-specific serum IgE (Th2 antibody) levels, but also lowered non-specific serum IgA (Th2 antibody) levels and OVA-specific IgG1 (Th2 antibody) levels. Obviously, dietary perilla oil differentially regulated Th2 antibody levels at the same time. However, it also failed to modulate Th1/Th2 antibody levels via isotype switching of B cells from Th2 antibody to Th1 antibody. The immunomodulatory property of perilla oil is consistent in cytokine secretion or antibody level profiles. Supportably, this work and the findings are identical to the previous study we done.

5. Conclusions

This study showed that dietary perilla oil lowered serum lipids and OVA-specific IgG1 levels in OVA-sensitized and -challenged mice. Although dietary perilla oil increased total IgE levels, it also lowered total IgA levels in sera. The results suggest that dietary perilla oil might have a moderately beneficial effect on asthma. However, it failed to obviously modulate Th1/Th2 antibody levels via isotype switching of B cells from Th2 antibody to Th1 antibody.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

- AIN, 1977. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* 107, 1340–1348.
- Albers, R., Bol, M., Bleumink, R., Willems, A., Blonk, C., Pieters, R., 2002. Effects of dietary lipids on immune function in a murine sensitization model. *Br. J. Nutr.* 88, 291–299.
- Al-Shawwa, B., Al-Huniti, N., Titus, G., Abu-Hasan, M., 2006. Hypercholesterolemia is a potential risk factor for asthma. *J. Asthma* 43, 231–233.
- Burdge, G.C., Calder, P.C., 2005. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 45, 581–597.
- Calder, P.C., 2005. Polyunsaturated fatty acids and inflammation. *Biochem. Soc. Trans.* 33, 423–427.
- Chang, H.-H., Chen, C.-S., Lin, J.-Y., 2008. Dietary perilla oil inhibits proinflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice. *Lipids* 43, 499–506.
- Connor, W.E., 2000. Importance of n-3 fatty acids in health and disease. *Am. J. Clin. Nutr.* 71 (Suppl.), 171S–175S.
- Engström, G., Lind, P., Hedblad, B., Stavenow, L., Janzon, L., Lindgärde, F., 2002. Effects of cholesterol and inflammation-sensitive plasma proteins on incidence of myocardial infarction and stroke in men. *Circulation* 105, 2632–2637.
- Harris, W.S., Miller, M., Tighe, A.P., Davidson, M.H., Schaefer, E.J., 2008. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis* 197, 12–24.
- Ide, T., Kobayashi, H., Ashakumary, L., Rouyer, I.A., Takahashi, Y., Aoyama, T., Hashimoto, T., Mizugaki, M., 2000. Comparative effects of perilla and fish oils on the activity and gene expression of fatty acid oxidation enzymes in rat liver. *Biochim. Biophys. Acta* 1485, 23–35.
- Javadi, M., Geelen, M.J.H., Lemmens, A.G., Lankhorst, A., Schonewille, J.T., Terpstra, A.H.M., Beynen, A.C., 2007. The influence of dietary linoleic and α -linolenic acid on body composition and the activities of key enzymes of hepatic lipogenesis and fatty acid oxidation in mice. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 91, 11–18.
- Kay, A.B., 2003. Immuno-modulation in asthma: mechanisms and possible pitfalls. *Curr. Opin. Pharmacol.* 3, 220–226.
- Kidd, P., 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* 8, 223–246.
- Kim, H.-K., Choi, H., 2001. Dietary α -linolenic acid lowers postprandial lipid levels with increase of eicosapentaenoic and docosahexaenoic acid contents in rat hepatic membrane. *Lipids* 36, 1331–1336.
- Kim, H.-K., Choi, S., Choi, H., 2004. Suppression of hepatic fatty acid synthase by feeding α -linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats. *J. Nutr. Biochem.* 15, 485–492.
- Kim, H.-K., Choi, H., 2005. Stimulation of acyl-CoA oxidase by α -linolenic acid-rich perilla oil lowers plasma triacylglycerol level in rats. *Life Sci.* 77, 1293–1306.
- Kurowska, E.M., Dresser, G.K., Deutsch, L., Vachon, D., Khalil, W., 2003. Bioavailability of omega-3 essential fatty acids from perilla seed oil. *Prostaglandins Leukot. Essent. Fatty Acids* 68, 207–212.
- Leyton, J., Drury, P.J., Crawford, M.A., 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* 51, 383–393.
- Lin, J.-Y., Chen, M.-L., Lin, B.-F., 2006a. *Ganoderma tsugae* in vivo modulates Th1/Th2 and macrophage responses in an allergic murine model. *Food Chem. Toxicol.* 44, 2025–2032.
- Lin, J.-Y., Lu, S., Liou, Y.-L., Liou, H.-L., 2006b. Increased IgA and IgG serum levels using a novel yam-boxthorn noodle in a BALB/c mouse model. *Food Chem. Toxicol.* 44, 170–178.
- Ma, D.W., Seo, J., Switzer, K.C., Fan, Y.Y., McMurray, D.N., Lupton, J.R., Chapkin, R.S., 2004. N-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. *J. Nutr. Biochem.* 15, 700–706.
- McKay, A., Leung, B.P., McInnes, I.B., Thomson, N.C., Liew, F.Y., 2004. A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. *J. Immunol.* 172, 2903–2908.
- Mickleborough, T.D., Rundell, K.W., 2005. Dietary polyunsaturated fatty acids in asthma- and exercise-induced broncho-constriction. *Eur. J. Clin. Nutr.* 59, 1335–1346.
- Morokata, T., Ishikawa, J., Yamada, T., 1999. Differential susceptibility of C57BL/6 and DBA/2 mice to ovalbumin-induced pulmonary eosinophilia regulated by Th1/Th2-type cytokines. *Immunol. Lett.* 70, 127–134.
- Nagai, T., Arai, Y., Emori, M., Nunome, S., Yabe, T., Takeda, T., Yamada, H., 2004. Anti-allergic activity of a Kampo (Japanese herbal) medicine “Sho-seiryu-to (Xiao-Qing-Long-Tang)” on airway inflammation in a mouse model. *Int. Immunopharmacol.* 4, 1353–1365.
- Opal, S.M., DePalo, V.A., 2000. Anti-inflammatory cytokines. *Chest* 117, 1162–1172.
- Otten, M.A., van Egmond, M., 2004. The Fc receptor for IgA (Fc α RI, CD89). *Immunol. Lett.* 92, 23–31.
- Rafalowski, R., Zegarska, Z., Kunciewicz, A., Borejszo, Z., 2008. Fatty acid composition, tocopherols and beta-carotene content in polish commercial vegetable oils. *Pakistan J. Nutr.* 7, 278–282.
- Scalia, R., Appel 3rd, J.Z., Lefer, A.M., 1998. Leukocyte-endothelium interaction during the early stages of hypercholesterolemia in the rabbit: role of P-selectin, ICAM-1, and VCAM-1. *Arterioscler. Thromb. Vasc. Biol.* 18, 1093–1100.
- Schmid-Grendelmeier, P., Fluckiger, S., Disch, R., Trautmann, A., Wuthrich, B., Blaser, K., Scheynius, A., Cramer, R., 2005. IgE mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. *J. Allergy Clin. Immunol.* 115, 1068–1075.
- Sijben, J.W.C., Calder, P.C., 2007. Differential immuno-modulation with long-chain n-3 PUFA in health and chronic disease. *Proc. Nutr. Soc.* 66, 237–259.
- Simopoulos, A.P., 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54, 438–463.
- Simopoulos, A.P., 2002a. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* 56, 365–379.
- Simopoulos, A.P., 2002b. Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* 21, 495–505.
- Stokes, K.Y., Cooper, D., Tailor, A., Granger, D.N., 2002. Hypercholesterolemia promotes inflammation and micro-vascular dysfunction: role of nitric oxide and superoxide. *Free Radic. Biol. Med.* 33, 1026–1036.
- Surette, M.E., Edens, M., Chilton, F.H., Trampusch, K.M., 2004. Dietary echium oil increases plasma and neutrophil long-chain (n-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. *J. Nutr.* 134, 1406–1411.
- Switzer, K.C., McMurray, D.N., Chapkin, R.S., 2004. Effects of dietary n-3 polyunsaturated fatty acids on T-cell membrane composition and function. *Lipids* 39, 1163–1170.
- Takano, H., Osakabe, N., Sanbongi, C., Yanagisawa, R., Inoue, K.-I., Yasuda, A., Natsume, M., Baba, S., Ichish, E.-I., Yoshikawa, T., 2004. Extract of *Perilla frutescens* enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. *Exp. Biol. Med.* 229, 247–254.
- Ueda, H., Yamazaki, C., Yamazaki, M., 2002. Luteolin as an anti-inflammatory and anti-allergic constituent of *Perilla frutescens*. *Biol. Pharm. Bull.* 25, 1197–1202.
- Wahrburg, U., 2004. What are the health effects of fat? *Eur. J. Clin. Nutr.* 43 (Suppl. 1), 1/6–1/11.

- Watanabe, S., Sakai, N., Yasui, Y., Kimura, Y., Kobayashi, T., Mizutani, T., Okuyama, H., 1994. A high α -linolenate diet suppresses antigen-induced immunoglobulin E response and anaphylactic shock in mice. *J. Nutr.* 124, 1566–1573.
- Woof, J.M., Kerr, M.A., 2004. IgA function – variations on a theme. *Immunology* 113, 175–177.
- Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., Okuyama, H., 1987. Effect of dietary α -linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* 28, 144–151.
- Yeh, Y.F., Huang, S.L., 2004. Enhancing effect of dietary cholesterol and inhibitory effect of pravastatin on allergic pulmonary inflammation. *J. Biomed. Sci.* 11, 599–606.