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Immunological and metabolic effects of *cis*-9, *trans*-11-conjugated linoleic acid in subjects with birch pollen allergy

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Animal studies suggest that conjugated linoleic acid (CLA) may modulate the immune response, while studies in healthy human subjects have shown little effect and results are controversial. However, the effects of CLA may be more prominent in situations of immune imbalance, such as allergy. We studied the effects of the natural CLA isomer, *cis*-9, *trans*-11-CLA, on allergy symptoms and immunological parameters in subjects with birch pollen allergy. In a randomised, placebo-controlled study, forty subjects (20–46 years) with diagnosed birch pollen allergy received 2 g CLA/d in capsules, which contained 65·3 % *cis*-9, *trans*-11-CLA and 8·5 % *trans*-10, *cis*-12-CLA (*n* 20), or placebo (high-oleic acid sunflower-seed oil) (*n* 20) for 12 weeks. The supplementation began 8 weeks before the birch pollen season and continued throughout the season. Allergy symptoms and use of medication were recorded daily. Lymphocyte subsets, cytokine production, immunoglobulins, C-reactive protein, lipid and glucose metabolism and lipid peroxidation were assessed before and after supplementation. The CLA group reported a better overall feeling of wellbeing ($P<0·05$) and less sneezing ($P<0·05$) during the pollen season. CLA supplementation decreased the *in vitro* production of TNF-α ($P<0·01$), interferon-γ ($P<0·05$) and IL-5 ($P<0·05$). Total plasma IgE and birch-specific IgE concentrations did not differ between groups, whereas plasma IgA ($P<0·05$), granulocyte macrophage colony-stimulating factor ($P<0·05$) and eosinophil-derived neurotoxin ($P<0·05$) concentrations were lower after CLA supplementation. Urinary excretion of 8-iso-$PGF_{2\alpha}$, a major F_2-isoprostane ($P<0·01$), and 15-keto-dihydro-$PGF_{2\alpha}$, a primary $PGF_{2\alpha}$ metabolite ($P<0·05$), increased in the CLA group. The results suggest that *cis*-9, *trans*-11-CLA has modest anti-inflammatory effects in allergic subjects.

Conjugated linoleic acid: Immune function: Cytokines: Immunoglobulin: Allergy: Lipid oxidation

Evidence from *in vitro* and animal studies suggests that conjugated linoleic acid (CLA), a group of positional and geometric conjugated dienoic isomers of linoleic acid, modulates immune function (for a review, see O'Shea *et al.* [1]). CLA has been shown to affect both humoral and cellular immune responses. Lymphocyte proliferation, immunoglobulin and cytokine production as well as proportion of T cell subtypes have been affected by CLA supplementation in animals[1].

CLA may also modulate the balance between Th1- and Th2-type immune responses by decreasing production of Th2 cytokines (IL-4, IL-5)[2–4] and by increasing production of Th1 cytokines (IL-2, interferon (IFN)-γ)[4–7]. In animals, CLA has shown inhibitory effects in type 1 hypersensitivity reactions[8,9]. CLA suppressed antigen-induced anaphylactic reactions in mice by inhibiting the decrease in blood pressure and blood flow[8]. Both orally and topically given CLA also suppressed scratching behaviour and vasodilation induced by a histamine release agent, compound 48/80[8]. In a guinea-pig tracheal superfusion model, CLA supplementation significantly reduced antigen-induced histamine and PGE_2 release[9].

In human subjects, effects of CLA have been modest. Antibodies to hepatitis B vaccination were increased in subjects supplemented daily with 1·7 g *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA (1:1), but no changes in cytokine production, lymphocyte proliferation, natural killer cell activity or delayed hypersensitivity were seen[10]. Effects of CLA did not differ from those of linoleic acid on lymphocyte proliferation and cytokine production in another study[11]. However, significant changes in immunoglobulin and cytokine production were observed in subjects fed 3 g CLA mixture (1:1 *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA) daily[12]. IgA and IgM production increased, while IgE, TNF-α and IFN-γ decreased[12]. Purified *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA isomers decreased mitogen-induced T lymphocyte activation dose-dependently, but did not affect *ex vivo* cytokine production[13].

All human studies on the effects of CLA on immune function have thus far been conducted in healthy subjects. The effects of CLA could be more profound in situations of immune imbalance, such as allergy. Thus, we conducted a study on the immunological and metabolic effects of *cis*-9, *trans*-11-CLA in subjects

Abbreviations: CLA, conjugated linoleic acid; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; PBMC, peripheral blood mononuclear cells.

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with birch pollen allergy. A CLA mixture containing predominantly the *cis*-9, *trans*-11 isomer was chosen since, should it prove beneficial, its dietary intake from dairy products and beef can be modulated by increasing the CLA content of these products through altered cow feeding regimens[14]. Also, adverse effects have been reported for the other major isomer, *trans*-10, *cis*-12-CLA[15–17].

Subjects and methods

Forty subjects (twenty-eight women and twelve men; age 20–46 years) with a diagnosis of birch pollen allergy were recruited from students and employees of the University of Helsinki. Health status was determined in screening tests. Exclusion criteria included chronic medication, consumption of dietary supplements, heavy smoking (more than ten cigarettes/d), heavy alcohol consumption (more than ten units per week), obesity (BMI $> 30\,kg/m^2$), pregnancy, lactation, diagnosis of CVD, diabetes, liver or endocrine dysfunction, asthma or other chronic inflammatory disease besides allergy. The selected subjects were normotensive (blood pressure $< 140/90$ mmHg), normolipidaemic (total cholesterol < 6 mmol/l, TAG concentration $< 2{\cdot}0$ mmol/l), normoglycaemic (blood glucose $< 6{\cdot}0$ mmol/l) and had no signs of acute inflammation at baseline (C-reactive protein < 10 mg/l).

A randomised, placebo-controlled study design was used. Subjects were assigned into CLA and placebo groups (twenty per group) and consumed three 1000 mg capsules daily for 12 weeks, providing 2 g *cis*-9, *trans*-11-CLA or high-oleic acid sunflower-seed oil. The CLA capsules contained 65·3 % *cis*-9, *trans*-11-CLA, 13·8 % 18 : 1*n*-9, 8·5 % *trans*-10, *cis*-12-CLA, 1·8 % *cis*-9, *cis*-12-18 : 2, 0·7 % *trans*-, *trans*-CLA and 5·4 % other fatty acids. The placebo capsules contained 78·1 % 18 : 1*n*-9, 12·7 % *cis*-9, *cis*-12-18 : 2, 0·1 % 18 : 3 and 8·6 % other fatty acids. Capsules were provided by Loders Croklaan, Lipid Nutrition (Wormerveer, The Netherlands).

Subjects were free living and were asked to maintain their normal dietary habits and physical activity. Allergy medication (antihistamine, eye drops, nasal spray) was provided during the birch pollen season, to be used when necessary for allergy symptoms. Subjects recorded allergy symptoms on a scale from 1 to 3 (1 = mild symptoms, 3 = severe symptoms) and use of allergy medication (as number of tablets, eye drops or sprays) in their study diaries daily.

Fasting blood samples were taken at baseline (week 0) and at the end of the pollen season (week 12). Morning urine samples were collected at the same time points.

Laboratory analyses

Blood count, lipids, glucose, C-reactive protein. Total blood count was performed on samples from week 0 and 12 using a Micros 60 blood analyser (ABX Diagnostics, Montpellier, France). Plasma lipid, glucose and C-reactive protein analyses were performed on a Konelab 20 autoanalyser (Thermo Fischer, Vantaa, Finland).

A commercial enzyme immunassay kit (Biosource, Nivelles, Belgium) was used to analyse plasma insulin concentrations. Samples were analysed in duplicate, with all samples of one subject on the sample plate. Control samples included in the kit were analysed with each batch of samples.

The homeostasis model for insulin resistance was calculated from fasting insulin and glucose concentrations $((\text{insulin}_0 \times \text{glucose}_0)/22{\cdot}5)$[18] and insulin sensitivity was calculated using the revised quantitative insulin sensitivity check index $(1/(\log \text{glucose}_0 + \log \text{insulin}_0 + \log \text{NEFA}_0))$[19].

Lymphocyte subsets. Proportions of CD4+ and CD8+ cells were determined from whole blood (100 μl) with fluorescent labelled mouse monoclonal antibodies against CD4 and CD8 (Serotec, Oxford, Oxon, UK). Samples were first incubated with the fluorescein isothiocyanate-conjugated antibody (10 μl) for 15 min at room temperature after which the erythrocytes were lysed with Cellkit C-04 (Cellset, Galmiz, Switzerland) and the cells were washed twice with PBS. Lymphocytes were fixed and the percentage of CD4+ and CD8+ cells was determined from a total of 10^4 cells with flow cytometry using a FACScan (Becton Dickinson, Franklin Lakes, NJ, USA).

Plasma immunoglobulins. Plasma concentrations of IgA, IgG and IgM were determined using an automated blood analyser (Konelab, Espoo, Finland). Total and birch-specific IgE concentrations were analysed using the Pharmacia CAP FEIA method (Pharmacia Diagnostics AB, Uppsala, Sweden).

Peripheral blood mononuclear cells stimulation. Peripheral blood mononuclear cells (PBMC) were isolated from 9 ml heparin blood by Ficoll-Paque centrifugation[20]. Blood was diluted with 10 % fetal calf serum–Roswell Park Memorial Institute (FCS/RPMI) culture media (1:1). Cells were counted in a Neubauer Improved counting chamber and diluted in the culture media to achieve a concentration of 2×10^6/ml. Cells were then frozen at −70°C.

PBMC were stimulated using birch pollen extract (ALK, Hørsholm, Denmark). Pollen extract was diluted using 10 % FCS/RPMI media to a final concentration of 10 000 standard quality (SQ)/ml.

Diluted antigen was pipetted into ninety-six-well microplates and cell suspensions (2×10^6/ml) were added to each well containing antigen or blank (10 % FCS/RPMI). Each sample was analysed in triplicate. The plates were incubated at 37°C and 5 % CO_2 for 6 d. Samples were centrifuged and supernatant fractions were collected. Supernatant fractions of replicate wells were pooled and stored at −70°C until analysed.

Cytokine production. *In vitro* production of IL-6 and TNF-α by stimulated PBMC were determined as general markers of inflammation. Plasma granulocyte macrophage colony-stimulating factor (GM-CSF), eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) concentrations as well as *in vitro* production of IL-4, IL-5, IL-10, IL-13 and IFN-γ by stimulated PBMC were analysed as markers of allergic inflammation. Plasma concentrations of EDN and ECP were determined using kits from MBL (Nagoya, Japan) according to the manufacturer's instructions. All other cytokines were analysed using ELISA kits from R&D Systems (Minneapolis, MN, USA). All samples were analysed in duplicate. Control samples included in each kit were measured with each batch of samples. The inter-assay and intra-assay CV were ≤ 10 % for all cytokines, excluding the inter-assay CV for IL-13 (11 %) and GM-CSF (15 %).

Lipid peroxidation. Urine samples collected before and after supplementation were analysed for free 8-iso-$PGF_{2\alpha}$, an indicator of free radical-mediated lipid peroxidation and 15-keto-dihydro-$PGF_{2\alpha}$, an indicator of cyclo-oxygenase-catalysed lipid peroxidation. Unextracted urine was analysed using highly specific

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and sensitive RIA for free 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ as previously described by Basu[21,22]. Urinary concentrations of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were adjusted for creatinine values measured with a commercial kit (IL test; Monarch Instrument, Amherst, NH, USA).

Statistical analysis

Data that were not normally distributed were log-transformed before testing. Differences between the two treatment groups were analysed with the unpaired *t* test. Spearman correlation tests were used to investigate relationships between the cytokines. Values of $P<0.05$ were considered statistically significant.

Results

Compliance

Thirty-eight subjects completed the study. One subject withdrew from the CLA group due to health problems considered unrelated to the supplement and one subject from the placebo group due to personal reasons.

Compliance according to capsule counting was 98 % in both groups. Also, analysis of plasma fatty acids indicated good compliance. In the CLA groups, the concentration of *cis*-9, *trans*-11-CLA increased from 0·20 % to 0·75 % of total fatty acids in plasma ($P<0.001$), while no change was seen in the placebo group (0·19 *v.* 0·22 %; $P=0.660$) (data not shown).

Allergy symptoms and medication

The CLA group reported a better overall feeling of wellbeing ($P<0.05$) (data not shown). There were no significant differences between groups in eye symptoms or total allergy symptoms (Fig. 1). Subjects in the CLA group reported significantly less sneezing ($P<0.05$) and tendency to less nasal symptoms ($P=0.07$). Use of medication did not differ between groups (data not shown) and correlated with allergy symptoms in both groups (r 0·698, $P=0.01$ for the CLA group and r 0·521, $P=0.02$ for the control group).

Blood count, lymphocyte subsets, lipids, glucose

No significant changes occurred in blood count, lymphocyte subsets, plasma lipids, glucose, insulin, homeostasis model for insulin resistance or quantitative insulin sensitivity check indices during the intervention (data not shown).

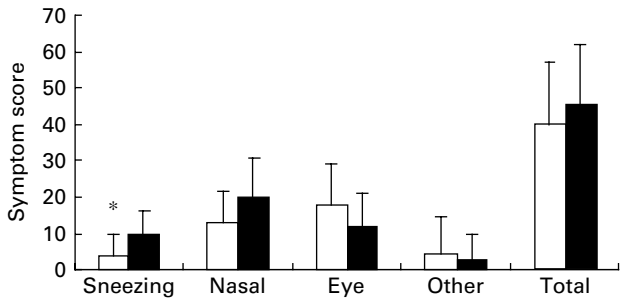

Fig. 1. Allergy symptoms in subjects supplemented with *cis*-9, *trans*-11-conjugated linoleic acid (□) or placebo (■). Values are means, with their standard deviations represented by vertical bars.

Plasma immunoglobulins

CLA supplementation significantly decreased plasma IgA concentrations ($P<0.01$) (Table 1). No changes in plasma IgG or IgM were observed. Concentrations of total and birch-specific IgE increased in both groups during the intervention. The increase in birch-specific IgE tended to be smaller in the CLA group ($P=0.07$).

In vitro *cytokine production*

Changes in the *in vitro* production of cytokines are presented in Fig. 2. The production of IL-4, IL-5, IL-6, IL-10, IL-13 and IFN-γ increased in both the CLA and placebo groups during the study. Production of TNF-α increased in the placebo group, but remained unchanged in the CLA group ($P<0.01$ for difference between groups).

IL-4 production increased 15–20-fold during the intervention, with no differences between groups.

IL-5 production was lower in the CLA group compared with placebo ($P<0.05$), whereas IL-10 production tended to be higher ($P=0.07$). Production of both IL-6 and IL-13 increased approximately twofold, with no differences between the CLA and placebo groups. Also IFN-γ increased in both groups, but the increase was greater in the placebo group ($P<0.05$ for difference between groups).

Several significant correlations between the cytokines were observed. IL-4 production was correlated with IL-5 (r 0·635; $P<0.001$) and IL-13 (r 0·470; $P<0.01$). IL-5 production was also correlated with IL-10 (r 0·635; $P<0.05$) and IL-13 (r 0·474; $P<0.01$). IL-6 and TNF-α were highly correlated (r 0·765; $P<0.01$). IFN-γ was correlated with IL-6 (r 0·459; $P<0.01$) and TNF-α (r 0·611; $P<0.001$).

TNF-α and IFN-γ were also correlated with sneezing and total symptoms, whereas no associations between IL-4, IL-5, IL-6, IL-10 or IL-13 and allergy symptoms were found.

Inflammatory markers and lipid peroxidation

Plasma C-reactive protein concentrations did not differ between groups (data not shown). However, a significant difference between groups in plasma GM-CSF was detected ($P<0.05$), as GM-CSF remained unchanged in the CLA group, but increased significantly ($P=0.02$) in the placebo group during the pollen season (Table 2). Changes in GM-CSF correlated with nasal symptoms (r 0·361; $P=0.031$), changes in IL-4 (r 0·361; $P=0.032$), IL-5 (r 0·440; $P=0.007$) and IFN-γ (r 0·558; $P<0.001$). Also plasma EDN concentrations increased significantly in the placebo group ($P=0.04$), but remained unchanged in the CLA group ($P=0.046$ for difference between groups) (Table 2). A similar tendency was seen in plasma ECP concentrations ($P=0.09$ for difference between groups).

On the contrary, urinary excretion of both 8-iso-$PGF_{2\alpha}$ ($P<0.01$) and 15-keto-dihydro-$PGF_{2\alpha}$ ($P<0.05$) increased significantly in the CLA group, but remained unchanged in the placebo group (Table 2). Changes in 8-iso-$PGF_{2\alpha}$ (r 0·467; $P=0.003$) and 15-keto-dihydro-$PGF_{2\alpha}$ (r 0·381; $P=0.020$) correlated with eye symptoms, whereas no correlations were found between isoprostane excretion and other allergy symptoms or cytokine production.

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Table 1. Plasma immunoglobulins in subjects supplemented with *cis*-9, *trans*-11-conjugated linoleic acid (CLA) or placebo (Mean values and standard deviations)

	Cis-9, *trans*-11-CLA (*n* 19)					Placebo (*n* 19)					
	March		May			March		May			
	Mean	SD	Mean	SD	Change	Mean	SD	Mean	SD	Change	*P**
IgA (g/l)	2·6	1·1	2·4	0·9	−0·2	2·2	0·5	2·3	0·7	0·1	0·006
IgG (g/l)	11·7	2·2	11·0	2·1	−0·7	11·9	1·7	11·6	1·7	−0·3	0·186
IgM (g/l)	1·1	0·5	1·1	0·5	0·0	1·0	0·5	1·1	0·4	0·1	0·309
Total IgE (kU/l)	64·1	37·5	81·4	36·6	17·3	80·6	55·5	104·0	62·4	25·9	0·093
Birch IgE (kU/l)	14·0	10·4	18·3	10·5	4·2	16·3	11·3	25·7	20·3	9·3	0·068

**P* for difference between the CLA and placebo groups.

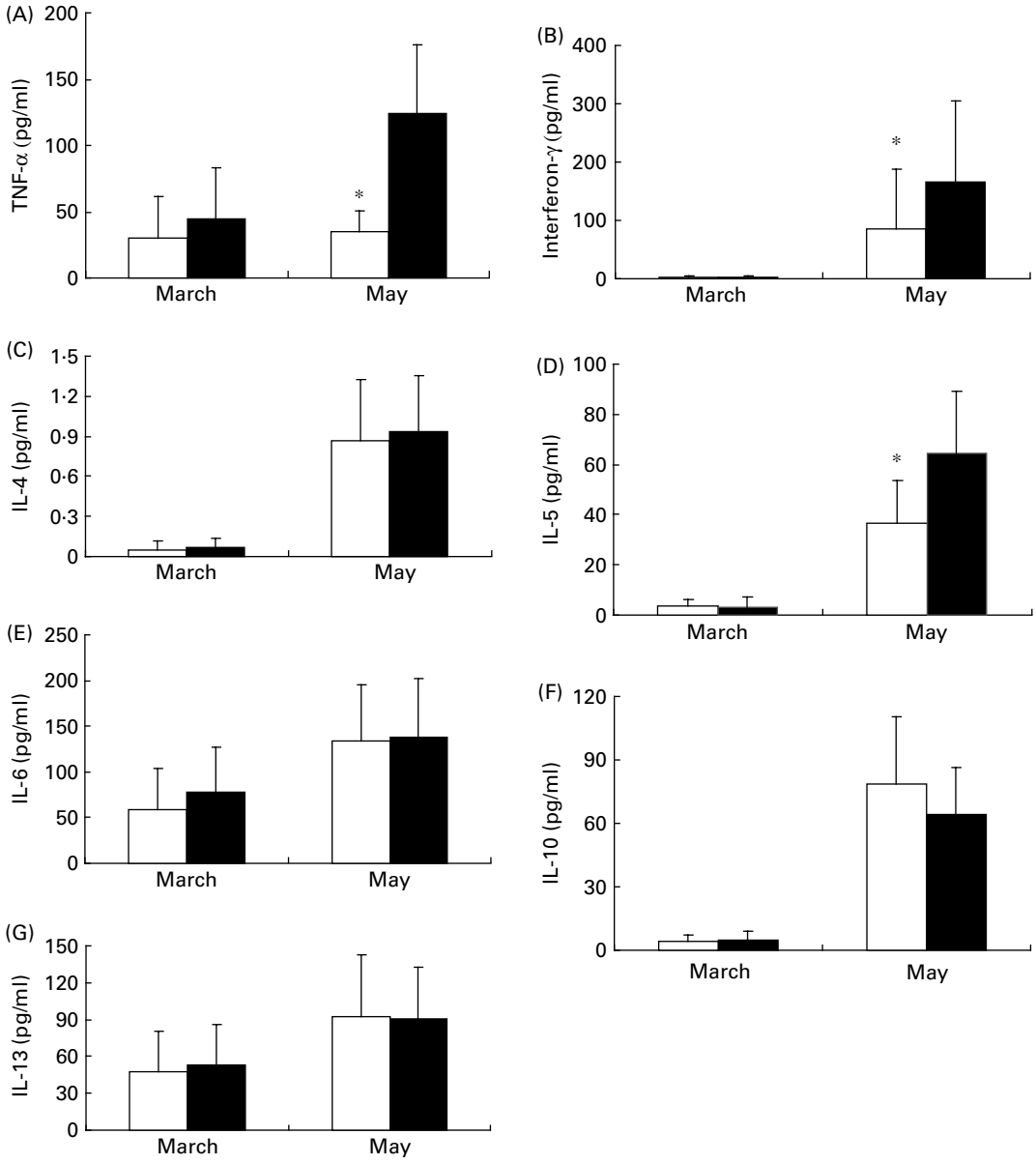

Fig. 2. *In vitro* production of TNF-α (A), interferon-γ (B), IL-4 (C), IL-5 (D), IL-6 (E), IL-10 (F) and IL-13 (G) by stimulated peripheral blood mononuclear cells in subjects supplemented with *cis*-9, *trans*-11-conjugated linoleic acid (□) or placebo (■). Values are means, with their standard deviations represented by vertical bars. * Mean value is significantly different from that of the placebo group ($P<0·05$).

Table 2. Effects of supplementation with *cis*-9, *trans*-11-conjugated linoleic acid (CLA) or placebo on plasma cytokine and urinary isoprostane concentrations

(Mean values and standard deviations)

	Cis-9, *trans*-11-CLA (*n* 19)					Placebo (*n* 19)					
	March		May			March		May			
	Mean	SD	Mean	SD	Change	Mean	SD	Mean	SD	Change	*P**
GM-CSF (pg/ml)	0·27	0·20	0·26	0·18	−0·01	0·19	0·15	0·35	0·28	0·16	0·041
ECP (ng/ml)	1·78	0·73	2·13	0·91	0·34	2·09	1·39	3·11	1·72	1·01	0·093
EDN (ng/ml)	19·6	10·4	18·7	8·3	−0·88	19·9	9·5	22·0	9·5	2·08	0·046
8-Iso-$PGF_{2\alpha}$ (nmol/mmol creatinine)	0·34	0·09	0·53	0·21	0·18	0·37	0·08	0·37	0·07	0·01	0·004
15-Keto-dOH-$PGF_{2\alpha}$ (nmol/mmol creatinine)	0·15	0·04	0·27	0·17	0·11	0·15	0·04	0·17	0·07	0·02	0·029

GM-CSF, granulocyte macrophage colony-stimulating factor; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin.
**P* for difference between the CLA and placebo groups.

A summary of the changes in allergy symptoms and immune responses observed in response to CLA supplementation is presented in Table 3.

Discussion

In our allergic subjects, *cis*-9, *trans*-11-CLA supplementation reduced sneezing, production of TNF-α, IFN-γ, IL-5, EDN and GM-CSF compared with high-oleic acid sunflower-seed oil. Previously, no data exist on the effects of CLA in allergic humans.

It is unclear why specifically sneezing was affected in our subjects, but it may be related to changes in cytokine production. Total allergy symptoms and sneezing correlated with the *in vitro* production of TNF-α and IFN-γ, both of which were reduced by CLA. Previously, scratching behaviour and vasodilation of the skin were inhibited in mice by topically applied CLA[8]. In subjects infected with rhinovirus, 2 g CLA/d supplementation (*cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA; 80:20) for 4 weeks before rhinovirus inoculation reduced coughing and sore throat compared with placebo[23]. Associations with cytokine production were not reported.

Table 3. Summary of the effects of conjugated linoleic acid (CLA) supplementation on allergy symptoms and immune responses in subjects with birch pollen allergy

	Effect of CLA supplementation
Total allergy symptoms	–
Sneezing	↓
Eye symptoms	–
Nasal symptoms	–
Total IgE	–
Birch-specific IgE	–
IL-4	–
IL-5	–
IL-6	–
IL-10	–
IL-13	–
TNF-α	↓
IFN-γ	↑
ECP	–
EDN	↓
GM-CSF	↓

–, No effect; ↓, decreased ($P<0·05$ for difference between CLA and placebo group); IFN, interferon; ↑, increased ($P<0·05$ for difference between CLA and placebo group); ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; GM-CSF, granulocyte macrophage colony-stimulating factor.

The decreased concentrations of TNF-α and IFN-γ suggest that *cis*-9, *trans*-11-CLA has inhibitory effects on Th1 cytokine production. TNF-α is an acute-phase protein and a key mediary in the local immune response. Decreased TNF-α in serum and tissues of animals has been reported after CLA supplementation in several studies[2,24–26]. Song *et al.* [12] also detected a 20 % decrease in TNF-α in healthy subjects supplemented with 3 g CLA mixture/d (1:1 *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA). However, Albers *et al.* [10] did not observe changes in TNF-α production following supplementation with 1·7 g CLA mixture/d in healthy subjects. Also, Tricon *et al.* [13] found no consistent effects for daily 0·6–2·5 g *cis*-9, *trans*-11-CLA or *trans*-10, *cis*-12-CLA on TNF-α production in healthy men. A possible explanation for the discrepancy between the studies is that due to the low baseline cytokine production in healthy subjects, significant changes are difficult to detect. Similarly, *n*-3 fatty acids have little effect in healthy subjects, whereas studies in different inflammatory conditions have shown dose-dependent anti-inflammatory effects for EPA and DHA[27].

IFN-γ has diverse immunoregulatory effects. It promotes Th1 responses, while inhibiting development of Th2 clones and IL-4-induced IgE synthesis[28]. However, recent evidence suggests that IFN-γ may also contribute to chronic inflammation[29,30]. In the present study, CLA supplementation decreased IFN-γ production. In line with the present results, Song *et al.* [12] also observed a 35 % decrease in IFN-γ in healthy subjects after daily supplementation with 3 g CLA mixture. In animals, CLA supplementation has increased IFN-γ production in healthy mice[4], but decreased IFN-γ in bacterial- or viral-induced inflammation in pigs[5,31]. Both in animals and in our subjects, the decrease in IFN-γ was also associated with decreased symptoms of the disease. The physiological significance of decreased IFN-γ in allergic subjects in response to CLA supplementation remains to be resolved.

Since eosinophil activation plays a major role in allergic inflammation, several factors affecting or indicating eosinophil activation were analysed. Of these, IL-5, GM-CSF and EDN were modulated by CLA supplementation in the present study, whereas IL-13 and ECP were not affected (Table 3). CLA has decreased IL-4 concentrations in animals[2–4], but effects on IL-5 and IL-13 have been reported rarely. Yamasaki *et al.* [32] supplemented C57BL/6J mice purified *cis*-9, *trans*-

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11-CLA, *trans*-10, *cis*-12-CLA, or a 50:50 blend of these two isomers for 3 weeks. Supplementation with the *cis*-9, *trans*-11-CLA isomer increased concanavalin A-stimulated TNF-α production, whereas no differences were observed between the groups with respect to IL-2, IL-4, IL-5 and IFN-γ production. In another study by the same group[4], the effects of CLA were dependent on the form of CLA: production of IL-5 and IL-13 was up regulated over 2-fold in splenocytes of mice fed 1 % CLA as TAG, but production of both was down regulated when the CLA was fed as NEFA as in the present study.

CLA supplementation inhibited the increase in EDN and GM-CSF concentrations seen in the placebo group during the pollen season. No correlations were seen between EDN and allergy symptoms, whereas changes in GM-CSF concentrations were correlated with nasal symptoms. Indications of an inhibitory effect of *cis*-9, *trans*-11-CLA on eosinophil activation were also found in a recent study by Jaudszus *et al.* [33]. *Cis*-9, *trans*-11-CLA, but not *trans*-10, *cis*-12-CLA, inhibited ECP production in a cytokine-stimulated co-culture of human eosinophils and bronchial epithelial cells[33]. Also, expression of eosinophil activation markers CD69 and CD13, which are induced by IL-5 and IL-13, was reduced by *cis*-9, *trans*-11-CLA[33]. Effects of CLA on GM-CSF or EDN have not been reported previously. Thus, more studies are needed to confirm our observations. Since local effects at the site of inflammation may be more pronounced than systemic effects, in the present study for example, analysis of nasal fluid could have given more insight into the effects of CLA on eosinophil activation and cytokine production.

In addition to total and birch-specific IgE, also IgA, IgG and IgM were measured, since previous studies both in animals and human subjects have shown that CLA modulates immunoglobulin production. CLA supplementation increased IgA, IgG and IgM production and decreased IgE production in rat spleen lymphocytes with only 0·1 % CLA in the diet[32,34,35]. In pigs, 0·5 % CLA increased IgG concentrations[36]. We, however, observed a decrease in IgA and a similar tendency for IgE in the CLA group, suggesting a suppressive effect on immunoglobulin production. The present results are also in contrast with Song *et al.* [12], who observed an increase in plasma IgA and IgM in healthy subjects fed 3 g CLA/d. A decrease in IgE was observed in both studies. The discrepancy between the present results and those of others may be due to the fact that in all other studies the CLA supplement contained *trans*-10, *cis*-12-CLA in nearly equal amounts as *cis*-9, *trans*-11-CLA.

An increase in free radical- and cyclo-oxygenase-mediated lipid oxidation after CLA supplementation has been observed in several studies[37–40]. In most studies, a CLA mixture containing nearly equal proportions but different doses of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers[16,37,38,40] or pure *trans*-10, *cis*-12-CLA[16] has been used, whereas also *cis*-9, *trans*-11-CLA (containing 7·3 % *trans*-10, *cis*-12-CLA) increased 8-iso-$PGF_{2\alpha}$ excretion by 50 % in obese men[39]. Previously, we detected an increase in urinary 8-iso-$PGF_{2\alpha}$ after dietary supplementation with vaccenic acid (*trans*-11-C18 : 1), a precursor of *cis*-9, *trans*-11-CLA[41]. A study using pure CLA isomers reported a significantly greater isoprostane production by the *trans*-10, *cis*-12-CLA isomer than *cis*-9, *trans*-11-CLA[16], which may reflect the different induction capacity of arachidonic acid oxidation by the two CLA isomers. Interestingly, the increase in lipid peroxidation in response to CLA (50 % for *cis*-9, *trans*-11-CLA; 578 % for *trans*-10, *cis*-12-CLA)[16,39] is of a different magnitude from the effect of other dietary fatty acids, for example, linoleic acid (42 % increase)[42] or fish oils (20–27 % increase)[43], and significantly greater than that caused by smoking (about 100 % increase), a strong inducer of oxidative stress[44]. Nevertheless, despite the induced free radical- and cyclo-oxygenase-mediated lipid oxidation, other inflammatory markers measured in the present study do not suggest a pro-inflammatory effect for *cis*-9, *trans*-11-CLA. Excretion of both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ correlated with eye symptoms, but this observation should be confirmed in other studies to determine whether an increase in lipid oxidation has clinical significance in allergic subjects.

The effects of CLA seen in the present study resemble the anti-inflammatory effects reported for long-chain *n*-3 fatty acids. EPA and DHA have decreased lymphocyte proliferation and cytokine production in a large number of studies[27]. The discrepancy between our observations in allergic subjects and reported effects of CLA in healthy subjects is also in line with findings with long-chain *n*-3 fatty acids, i.e. healthy subjects are rather insensitive to the effects of *n*-3 fatty acids, whereas effects are more profound in different inflammatory conditions[27]. Evidence regarding the immune-modulating effects of *n*-6 fatty acids is more controversial. Linoleic acid is generally considered pro-inflammatory as it is metabolised into arachidonic acid, which in turn is the precursor of the pro-inflammatory eicosanoids PGE_2 and leucotriene B_4. However, another *n*-6 fatty acid, γ-linolenic acid, has been associated with anti-inflammatory effects mainly in atopic dermatitis, but the evidence is not consistent[45].

We observed inhibitory effects on both Th1 and Th2 responses and specifically eosinophil activation, whereas some established markers of allergy (IgE, IL-4) were not affected (Table 3). Recent studies suggest that both Th1 and Th2 responses are increased in allergic subjects[46,47], contrary to an earlier hypothesis of an impaired Th1 function and a Th2 skewing effect. Thus, suppression of both Th1 and Th2 cytokine responses seen in the present study suggest a general anti-inflammatory effect for *cis*-9, *trans*-11-CLA. The effects of CLA could be mediated via several mechanisms of action. Yu *et al.* [48] and Jaudszus *et al.* [33] reported that the decrease in pro-inflammatory cytokines (TNF-α, IL-6, IL-8) by CLA was mediated via PPARγ activation. CLA may also modulate cytokine synthesis by directly affecting gene expression of cytokines[49] or via changes in eicosanoid synthesis and signalling[49,50]. As the present study is the first to study effects of *cis*-9, *trans*-11-CLA on immune responses in allergic subjects, more studies in allergy and asthma are warranted to confirm our observations.

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