Dietary supplementation with trans-11- and trans-12-18:1 increases cis-9, trans-11-conjugated linoleic acid in human immune cells, but without effects on biomarkers of immune function and inflammation

Katrin Kuhnt¹, Jana Kraft¹, Heinz Vogelsang², Klaus Eder³, Jürgen Kratzsch⁴ and Gerhard Jahreis¹*  
¹Institute of Nutrition, Friedrich Schiller University, Dornburger Strasse 24, D-07743 Jena, Germany  
²Institute of Clinical Chemistry and Laboratory Diagnostics, Friedrich Schiller University, Jena, Germany  
³Institute of Nutrition, Martin Luther University, Halle/Saale, Germany  
⁴Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Germany  
(Received 19 September 2006 – Revised 20 December 2006 – Accepted 3 January 2007)

Trans-fatty acid intake is associated with an increased risk of CHD and diabetes. The effects of single trans-fatty acid isomers are largely unexplored. The present study examined the effects of a 6-week supplementation with two trans-18:1 isomers (trans-11 and trans-12) in human subjects on immune cells, several inflammatory and immunological biomarkers (for example, IL, TNFα, C-reactive protein, adiponectin, intercellular adhesion molecule-1, prostacyclin, phagocytic process). Following a 2-week adaptation period without supplements, the test group (n = 12) received vaccenic acid (trans-11-18:1) and trans-12-18:1 in equal amounts (6 g/d) for 6 weeks. The control group (n = 12) consumed an oil without trans-fatty acids and conjugated linoleic acids (CLA). Samples were collected at the end of both periods. Trans-11- and trans-12-18:1 were significantly increased in cellular lipids. The endogenous synthesis of cis-9, trans-11-CLA from trans-11-18:1 was demonstrated via increased CLA in cellular lipids of the test group. Generally, trans-isomer supplementation did not affect either inflammatory biomarkers (for example, IL-6, IL-8, TNFα) or immune function (for example, phagocytosis) during the present study. The dietary supplementation of trans-11- and trans-12-18:1 (6 g/d) and their accumulation in leucocytes had no effects on biomarkers of inflammation and immune function. However, because of the limited data on the safety of trans-fatty acid intake and effects of individual trans isomers on human health (for example, trans-9-18:1, trans-10-18:1) at present, it is prudent to reduce trans-fat intake in general.

Trans-fatty acids: Conjugated linoleic acid: Inflammation: Immune function

Trans-fat is a class of unsaturated fatty acids that possess at least one double bond in the trans configuration. The most common trans-fatty acids in the diet are trans-octadecenoic acids (18:1; Steinhart et al. 2003), consisting of a large number of positional isomers (trans-4 to trans-16). Ruminant-derived products (milk and meat) contain trans-fatty acids in smaller quantities (1–8 % fatty acids, with vaccenic acid (trans-11-18:1) as the major trans isomer) than partially hydrogenated fats and industrially prepared food (up to 60 % fatty acids with trans-9- and trans-10-18:1 as the major trans isomers; Aro et al. 1998; Craig-Schmidt, 1998).

Conjugated linoleic acids (CLA) refer to a group of geometrical and positional isomers of linoleic acid (Delmonte et al. 2004). The most abundant naturally occurring CLA isomer is the cis-9, trans-11 (c9,t11)-CLA which is widely found in ruminant-related products (Kraft et al. 2003). It is formed both by anaerobic biohydrogenation of linoleic acid in the rumen (Bauman & Grinari, 2003), but mainly by endogenous Δ9-desaturation (via stearoyl-CoA desaturase (SCD); EC 1·14·99·5) in the mammary gland and other tissues with trans-11-18:1 as the precursor (Mosley et al. 2006). This endogenous CLA synthesis has also been observed in non-ruminant animals and human subjects (Turpeinen et al. 2002; Kraft et al. 2006; Kuhnt et al. 2006a).

The average daily intake of trans-fatty acids is higher in US and Canadian populations (about 5·8 g/d; 2·6 % energy intake; Food & Drug Administration, 2003, 2006) than in European populations (about 2·2 g/d; 0·9 % energy intake; van de Vijver et al. 2000). Interestingly, in the USA and Canada approximately 80 % of total trans-fatty acids are currently derived from industrially processed food products containing hydrogenated vegetable oils. In contrast, in the European Union about 40 % are derived from hydrogenated vegetable oils. In the European Union, the intake of total trans-18:1 from ruminant fats was estimated to be from 1·3 to 1·8 g/d (Wolff, 1995). Thus, trans-11-18:1 intake was estimated at 1·0 g/d whereas CLA intake was lower and ranged between 0·1 and 0·5 g/d (Fremann et al. 2002; Jahreis & Kraft, 2002).

Abbreviations: c9,t11, cis-9, trans-11; CLA, conjugated linoleic acid; CRP, C-reactive protein; FAME, fatty acid methyl esters; fMLP, N-formyl-Met-Leu-Phe; ICAM, intercellular adhesion molecule; 6-keto-PGF 1α, 6-keto-prostaglandin F 1α; PGI 2, prostacyclin; PBMC, peripheral blood mononuclear cells; SCD, stearoyl-CoA desaturase; sPLA 2, secretory phospholipase A 2.

* Corresponding author: Dr Gerhard Jahreis, fax +49 3641 949612, email b6jage@uni-jena.de
The impact of dietary trans-fatty acids and CLA on inflammatory processes and on the immune system in human subjects requires further evaluation. Trans-fatty acid intake has been related to endothelial dysfunction (Lopez-Garcia et al. 2005), inflammation (Mozaffarian et al. 2004a,b), type 2 diabetes (Bray et al. 2002; Lefevre et al. 2005) and to an increased risk of CVD (Lemaitre et al. 2006; Mensink et al. 2003; Mozaffarian et al. 2006). Several studies have shown that trans-fatty acids affect plasma markers of inflammation, such as pro-inflammatory cytokines for example, IL-6, TNFα, acute-phase proteins (for example, C-reactive protein (CRP)), and adhesion molecules (for example, intercellular adhesion molecule (ICAM)-1) (Baer et al. 2004; Lopez-Garcia et al. 2005).

In contrast to trans-fatty acids, CLA (for example, isomer dependent; c9,t11 and trans-10, cis-12) were found in cell and animal studies to have anti-inflammatory activity (suppressing eicosanoid synthesis; for example, prostaglandin E2 and prostaglandin I2 (prostacyclin; PGI2) (Bulgarella 2006) and diets have been described in detail previously (Kuhnt et al. 2001; Alders et al. 2003; Tricon et al. 2004).

The present study was designed to investigate the effects of a 6-week dietary supplementation of 3.0 g trans-11-18 : 1 and 3.0 g trans-12-18 : 1 and endogenous CLA synthesis on several biomarkers (for example, IL-6, 8, TNFα, CRP, ICAM-1, leptin, adiponectin, N metabolites, PGI2, activity of phospholipase A2, and transaminases). In addition, we determined the fatty acid composition and the intervention period of trans-11-18 : 1 and trans-12-18 : 1 and their Δ9-desaturation products (c9,t11-CLA and cis-9, trans-12-18 : 2) into lipids of peripheral blood mononuclear cells (PBMC) and the phagocytic activity of granulocytes.

**Subjects and methods**

**Subjects and diets**

The study was approved by the ethics committee of the Friedrich Schiller University of Jena (Germany). The study design and diets have been described in detail previously (Kuhnt et al. 2006a). Twenty-four healthy subjects participated in the present study (Table 1). Throughout the entire study (8 weeks) the consumed basal diet of each subject had to contain only marginal amounts of trans-fatty acids and CLA. The subjects received written instructions to keep the conditions of the trans-fatty acid-free and CLA-free basal diet.

The subjects were randomly assigned and divided into the control group and the test group (each group, n 12). Each study group consisted of six men and six women. The study started with a 2-week adaptation period (baseline) without supplementation. During this period all volunteers consumed daily 20 g pure commercial chocolate spread (% fatty acid methyl esters (FAME): 18 : 1, 60%; 16 : 0, 18%; 18 : 2, 13%) to make the adaptation diet isoenergetic compared with the intervention diet. During the intervention period the diet of the test group was supplemented with 3.0 g trans-11-18 : 1/d and 3.0 g trans-12-18 : 1/d (% FAME in trans-isomer mixture: trans-11- and trans-12-18 : 1, 60%; cis-11- and cis-12-18 : 1, 20%; 18 : 0, 11%; Natural ASA, Hovdebygda, Norway). The diet of the control group was supplemented with control oil free of CLA and trans-fatty acids to make the intervention diets isoenergetic. The control oil was a mixture of palm kernel oil and rapeseed oil (1 : 1) with a fatty acid distribution almost similar to the chocolate spread (% FAME: 18 : 1, 50%; 16 : 0, 14%; 18 : 2, 12%).

In order to standardise the dietary food before blood collection all subjects received fresh food every day from our department during the last week of both study periods (Table 2). Both preparations (control oil and trans-isomer mixture) were added to chocolate spread to achieve a good acceptability during the intervention period. Each subject consumed daily 20 g chocolate spread enriched with the trans-isomer mixture or the control oil.

**Blood sampling**

Blood samples were collected on the last day of the standardised diet of the adaptation period (baseline; day 0) and the intervention period (day 42). Blood samples were taken between 07:30 and 08:30 hours after overnight fasting by venepuncture into EDTA-vacutainer® tubes (BD Vacutainer Systems, Heidelberg, Germany). In addition, for eicosanoid determination the EDTA blood was mixed immediately with indomethacin (0.5 mmol/ml distilled water; Sigma-Aldrich, St Louis, MO, USA), an inhibitor for cyclooxygenase.

**Preparation of peripheral blood mononuclear cells**

Fresh EDTA blood was diluted 1 : 1 with PBS. The diluted blood was layered carefully onto Histopaque® (density 1.077 g/l, diluted blood: Histopaque ratio was 4 : 3; Sigma-Aldrich, Munich, Germany) and centrifuged for 30 min at 400 g at 20°C. The uppermost layer of fluid (plasma) was removed and then the opaque PBMC layer (mixture of monocytes and lymphocytes) was collected from the interphase. The PBMC were washed with PBS twice (centrifugation at 250 g,
10 min) to lower the degree of erythrocyte contamination. Cell count was determined using a Neubauer haemocytometer counting chamber (Roth, Karlsruhe, Germany).

Analysis of lipids of peripheral blood mononuclear cells

The detailed procedures and results of lipid analysis have been previously described (Kuhnt et al. 2006a). Briefly, total lipids of PBMC (at least 20 × 10⁶) were extracted with chloroform–methanol–water (2:1:1, by vol.). Tricosanoate (TAG, C23:0) was added to each lipid extract as an internal standard. FAME were prepared with 1,1,3,3-tetramethylguanidine in methanol (1:4, v/v, 5 min, 100°C; Sigma-Aldrich) and purified by TLC on silica gel plates (Merck, Darmstadt, Germany). FAME were separated by two different GC procedures (GC-17 V3; Shimadzu, Kyoto, Japan) and detected with a flame ionisation detector. The first GC procedure determined the fatty acid distribution of the CLA isomers was determined using Ag⁺-HPLC (LC10A; Shimadzu) according to Kraft et al. (2003). Fatty acids were identified by comparison with standard FAME (Sigma-Aldrich and Larodan, Malmö, Sweden) run previously.

Immuno phenotyping

The two-colour immunophenotyping was conducted by flow cytometry in a flow cytometer FACScan™ employing simulset™ software, simultest™ IMK-Lymphocyte test kit, and several different fluorochrome-labelled monoclonal antibodies (BD Biosciences, Heidelberg, Germany). The percentage of lymphocytes, monocytes, and granulocytes of total leucocytes (CD45 carrying cells) was determined by using CD14/CD45 gating. The fluorochrome-labelled monoclonal antibodies utilised in the subpopulations of leucocytes determinations included: total T (CD3⁺) lymphocytes, B (CD19⁺) lymphocytes, helper/inducer T (CD3⁺CD4⁺) lymphocytes, suppressor/cytotoxic T (CD3⁺CD8⁺) lymphocytes, natural killer lymphocytes (identified as CD3⁺CD16⁺ and/or CD56⁺) and several subsets of lymphocytes such as the activated T (CD3⁺/HLA-DR⁺) lymphocytes, CD25 (α-chain of the IL-2-receptor), CD4⁺CD25⁺ (helper cell carrying IL-2 receptor), CD54 (ICAM-1) and CD130 (IL-6 receptor-associated signal transducer).
Phagocytic process

The quantitative analysis of leucocyte phagocytosis in human blood was conducted as an ex vivo multifactorial process according to the manufacturers’ instructions for the various required testing assays: Migratest® to measure chemotaxis, Phagotest® to measure ingestion of microbes, and Phagoburst® to measure oxidative burst (ORPEGEN Pharma, Heidelberg, Germany). The cell preparations were analysed by flow cytometry (FACScan™; BD Biosciences, San Jose, CA, USA) and fluorescence data were analysed with the use of CELLQUEST™ software (BD Biosciences). The Migrat-test® allows the quantitative determination of the chemotactic activity of neutrophilic granulocytes which have migrated through a membrane (pore size 3.0 μm) towards a gradient of the chemotaxtractant N-formyl-Met-Leu-Phe (fMLP). In addition, the expression of leucocyte-endothelial cell adhesion molecule-1 and the cell shape change with the forward scatter signals were determined. These measurements were conducted under fMLP (stimulated positive test samples, +fMLP) conditions and compared with incubation buffer (negative control, −fMLP) conditions. The Phagotest® and Phagoburst® measured the percentage of neutrophilic granulocytes which demonstrated phagocytosis (ingestion of bacteria) and oxidative burst rates (intracellular killing by O₂-dependent mechanisms). The median fluorescence intensity enabled the measurement of the number of ingested bacteria per cell and burst activity per cell.

Cytokines

Increases in plasma concentrations of various soluble cytokines (IL-1β, IL-6, TNFα) and TNFα are indicators of inflammation. These plasma factors were analysed via a human inflammation Cytometric Bead Array kit using flow cytometry (FACScan™ instruments and CELLQUEST™ software; BD Biosciences). Samples were analysed as triplicates. Intra-assay and inter-assay CV of IL-1β, IL-6, and TNFα were lower than 13 % (69–78 pg/ml).

Adipokines

Adipose tissue secretes a variety of biologically active molecules, adipokines, such as leptin and adiponectin. Plasma concentration of leptin was measured using an in-house RIA as described previously (Kratzsch et al. 2002). Adiponectin concentration was also measured by RIA (Linco Research, St Charles, MO, USA). Samples were analysed as duplicates. Intra-assay and inter-assay CV of leptin and adiponectin were 12.5 % (5 ng/ml) and 9.6 % (6 ng/ml), respectively.

Prostacyclin and secretory phospholipase A₂ activity

The effects of the trans-11- and trans-12-18 : 1 supplementation on secretory phospholipase A₂ (sPLA₂) activity in plasma were assessed using an sPLA₂ assay kit (Cayman Chemical, Ann Arbor, MI, USA). PGJ₂, an endothelial prostaglandin, is quickly hydrated to its more stable metabolite 6-keto-prostaglandin F₁α (6-keto-PGF₁α). The plasma 6-keto-PGF₁α metabolite concentrations were utilised to estimate PGJ₂ concentrations and were analysed by an EIA kit (Cayman Chemical). Samples were analysed as triplicates and intra-assay and inter-assay CV of 6-keto-PGF₁α were lower than 15 % (50 pg/ml).

Activity of transferases and the concentrations of creatinine, bilirubin, uric acid, urea, and C-reactive protein in plasma

The activity of several transferases, specific for liver injury (γ-glutamyltransferase (EC 2.3.2.2), aspartate aminotransferase (EC 2.6.1-1), alanine aminotransferase (EC 2.6.1-2)), and plasma concentrations of total bilirubin, creatinine, uric acid and urea were determined by enzymic assays using the Synchron LX®-20-system (Beckman Coulter, Fullerton, CA, USA) according to the methods of the International Federation of Clinical Chemistry and Laboratory Medicine. As an indicator of acute inflammation, CRP concentration was quantified by using a turbidimetric immunoaassay assay on the Synchron LX®-20-system (Beckman Coulter).

Statistical analysis

All statistical analyses were performed using SPSS software package, version 11.5 (SPSS Inc., Chicago, IL, USA). The P value ≤ 0.05 was regarded as significant. Values are reported as mean values and standard deviations. Sex-related baseline data were compared using the t test. The Kolmogorov–Smirnov test was used to test the distribution of the data. All measures were normally distributed. Data analyses were conducted as two-factor (sex and diets) ANOVA with interaction. Analysis of covariance (baseline as covariate) was used to compare data of the two treatments. Correlations were calculated by using Pearson correlation analysis.

Results

Fatty acid distribution of peripheral blood mononuclear cells

Trans-11- and trans-12-18 : 1 were incorporated into the membrane lipids of PBMC. Trans-12-18 : 1 was more readily incorporated than trans-11-18 : 1 (Table 3). cis,11-CLA was also significantly increased. Despite the elevated trans-12-18 : 1 content in membrane lipids of PBMC, the cis-9, trans-12-18 : 2 remained unchanged. After the intervention period, the 22 : 6n-3 proportion of the test groups’ PBMC membrane lipids was significantly lower than that of the control group. Other fatty acids were not affected (Table 3).

Clinical, immunological, and inflammatory parameters

In general, the trans-isomer treatment produced no significant differences in the clinical, immunological and inflammatory parameters analysed for the two treatment groups. No treatment effects were shown on sex subgroups (Tables 4 and 5).

Phagocytic process

The examination of the phagocytic process of granulocytes included their migration, ingestion and oxidative burst rates. No significant differences in the number of chemotactic cells after stimulation were observed between the study groups after supplementation of the intervention treatments (control
Table 3. The effects of dietary supplementation of trans-11- and trans-12-18:1 isomers (6 g/d; 1:1) on the fatty acid profile of human peripheral blood mononuclear cells lipids (% total fatty acid methyl esters) (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control group (n=12)</th>
<th>Test group (n=12)</th>
<th>Control group (n=12)</th>
<th>Test group (n=12)</th>
<th>Treatment effect† (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>19.84 (1.64)</td>
<td>19.28 (3.43)</td>
<td>20.06 (2.13)</td>
<td>19.79 (2.62)</td>
<td>NS</td>
</tr>
<tr>
<td>cis-9:16:1</td>
<td>0.86 (0.07)</td>
<td>0.79 (0.14)</td>
<td>0.81 (0.09)</td>
<td>0.85 (0.14)</td>
<td>NS</td>
</tr>
<tr>
<td>18:0</td>
<td>22.34 (1.22)</td>
<td>21.40 (2.74)</td>
<td>22.16 (1.43)</td>
<td>20.91 (2.34)</td>
<td>NS</td>
</tr>
<tr>
<td>cis-9:18:1</td>
<td>15.32 (0.83)</td>
<td>14.80 (1.61)</td>
<td>15.53 (1.75)</td>
<td>16.13 (1.71)</td>
<td>NS</td>
</tr>
<tr>
<td>trans-11:18:1</td>
<td>0.05 (0.03)</td>
<td>0.06 (0.05)</td>
<td>0.05 (0.04)</td>
<td>0.45 (0.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>trans-12:18:1</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.83 (0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cis-9, cis-12:18:2</td>
<td>6.99 (0.43)</td>
<td>6.76 (0.91)</td>
<td>6.97 (0.76)</td>
<td>6.61 (0.56)</td>
<td>NS</td>
</tr>
<tr>
<td>cis-9, trans-11:18:2</td>
<td>0.07 (0.00)</td>
<td>0.08 (0.01)</td>
<td>0.07 (0.00)</td>
<td>0.07 (0.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>11.67 (1.90)</td>
<td>11.19 (1.57)</td>
<td>11.79 (2.82)</td>
<td>11.11 (2.85)</td>
<td>NS</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.12 (0.03)</td>
<td>0.18 (0.05)</td>
<td>0.12 (0.04)</td>
<td>0.16 (0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.53 (0.32)</td>
<td>0.49 (0.27)</td>
<td>0.54 (0.36)</td>
<td>0.33 (0.12)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* No significant treatment x sex interactions.
† Significantly different from the control group with baseline value as covariate.
‡ Conjugated linoleic acid.

Plasma concentrations of nitrogen metabolites, C-reactive protein and the activity of transferases

The plasma concentrations of N metabolites (total bilirubin, urea, uric acid and creatinine) did not significantly differ when the treatment groups were compared (Table 5). The concentrations of urea, uric acid and creatinine were positively correlated with total bilirubin in both groups after baseline adaptation.

Table 4. The effects of the dietary supplementation of trans-11- and trans-12-18:1 isomers (6 g/d; 1:1) on the circulating immune cells and subtypes of lymphocytes (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Adaptation period (day 0)</th>
<th>Intervention period (day 42)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test group (n=12)</td>
</tr>
<tr>
<td>Total leucocytes (CD45-carrying cells) (%)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>38.6 (8.8)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6.7 (1.0)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>54.7 (8.7)</td>
</tr>
<tr>
<td>Total lymphocytes (%)</td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td>68.8 (7.1)</td>
</tr>
<tr>
<td>CD3+/CD4+</td>
<td>40.3 (4.8)</td>
</tr>
<tr>
<td>CD3+/CD8+</td>
<td>23.8 (5.7)</td>
</tr>
<tr>
<td>CD4+CD8+</td>
<td>1.9 (0.7)</td>
</tr>
<tr>
<td>CD19+</td>
<td>11.2 (3.2)</td>
</tr>
<tr>
<td>CD3+CD16+/CD56+</td>
<td>16.1 (6.4)</td>
</tr>
<tr>
<td>CD3+HLA-DR+</td>
<td>4.4 (1.1)</td>
</tr>
<tr>
<td>CD57+</td>
<td>11.4 (4.1)</td>
</tr>
<tr>
<td>CD8+CD57</td>
<td>5.1 (2.1)</td>
</tr>
<tr>
<td>CD25</td>
<td>19.8 (3.3)</td>
</tr>
<tr>
<td>CD4+CD25+</td>
<td>13.8 (3.6)</td>
</tr>
<tr>
<td>CD54</td>
<td>59.4 (8.7)</td>
</tr>
<tr>
<td>CD4+CD54+</td>
<td>12.3 (3.5)</td>
</tr>
<tr>
<td>CD130</td>
<td>41.7 (8.6)</td>
</tr>
<tr>
<td>CD4+CD130+</td>
<td>26.1 (5.3)</td>
</tr>
</tbody>
</table>

CD, cluster of differentiation.
* No significant treatment x sex interactions; no significant differences between the control and test groups with baseline value as covariate.
and intervention periods (data not shown; $P=0.017$). Furthermore, the concentration of urea correlated with uric acid (control group $r=0.614$, $P=0.034$; test group $r=0.399$, $P=0.199$) and creatinine (control group $r=0.586$, $P=0.045$; test group $r=0.709$, $P=0.010$). The plasma concentration of CRP did not exceed 3 mg/l.

A decrease in the activity of plasma γ-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase was observed after the intervention period. However, no significant differences between the treatment groups were demonstrated. The activity of these enzymes correlated positively with each other during both study periods (data not shown).

No significant differences in the plasma concentrations of cytokines in both treatment groups were unaffected by the intervention treatment (Table 5). There were significant correlations between IL-6 and TNFα (adaptation period: control group $r=0.813$, $P=0.001$; test group $r=0.634$, $P=0.027$) and IL-8 and TNFα (intervention period: control group $r=0.562$, $P=0.057$; test group $r=0.763$, $P=0.004$).

No significant differences in the plasma concentrations of leptin and adiponectin were observed (Table 5). Sex differences were observed in plasma leptin concentrations that were independent of the treatment group; female subjects possessed higher plasma leptin concentrations than their male counterparts (female 11·3 (SD 4·6) ng/ml v. male 2·3 (SD 2·1) ng/ml; $P=0.007$). No significant correlation between plasma adiponectin and leptin was observed. The correlation between plasma adiponectin and leptin was negative but not significant for both treatment groups during both study periods (data not shown).

Another sex difference was observed in the percentage of body fat (BIA 2000-C; Data Input GmbH, Darmstadt, Germany); female subjects demonstrated a significantly higher percentage of body fat than their male counterparts independent of the treatment group (adaptation period 22·3 (SD 4·7) v. 15·8 (SD 5·8) %; intervention period 21·6 (SD 5·0) v. 14·8 (SD 5·8) %). The plasma leptin concentration was positively correlated with body fat in both sexes (adaptation period: male $r=0.848$, $P<0.001$; female $r=0.774$, $P=0.005$; intervention period: male $r=0.786$, $P=0.002$; female $r=0.779$, $P=0.005$). The plasma adiponectin concentration correlated negatively with body fat in both sexes of both study periods, but without significance (data not shown).

The activity of the sPLA2 and the plasma concentration of 6-keto-PGF1α were not different between the treatment groups.

### Discussion

The incorporation of fatty acids into cellular lipids can influence their physiological functions (Kew et al. 2003, 2004). Trans-fatty acid intake is positively associated with inflammation and increased insulin resistance in human subjects (Baer et al. 2004; Mozaffarian et al. 2004a). Systemic inflammation has been reported as an independent risk factor for heart disease (Libby, 2002). Therefore, changes in the long-term trans-fatty acid concentrations in human tissues result in changes to the risk of developing and/or to the rate

<table>
<thead>
<tr>
<th>Plasma biomarker</th>
<th>Adaptation period (day 0)</th>
<th>Intervention period (day 42)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n 12)</td>
<td>Test group (n 12)</td>
</tr>
<tr>
<td>Bilirubin (μmol/l)</td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>154</td>
<td>54</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>γ-GT activity (μmol/s)</td>
<td>0·32</td>
<td>0·07</td>
</tr>
<tr>
<td>ALAT activity (μmol/l per s)</td>
<td>0·31</td>
<td>0·16</td>
</tr>
<tr>
<td>ASAT activity (μmol/l per s)</td>
<td>0·41</td>
<td>0·14</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>3.93</td>
<td>1.88</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>38.11</td>
<td>36.42</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.83</td>
<td>1.45</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>37.71</td>
<td>32.36</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>7.73</td>
<td>4.64</td>
</tr>
<tr>
<td>IL-12-p70 (pg/ml)</td>
<td>12.87</td>
<td>8.56</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.82</td>
<td>3.86</td>
</tr>
<tr>
<td>Adiponectin (mg/ml)</td>
<td>7.26</td>
<td>2.26</td>
</tr>
<tr>
<td>sPLA2 activity (pmol/min per l)</td>
<td>0.26</td>
<td>0.07</td>
</tr>
<tr>
<td>6-Keto-PGF1α (pg/ml)</td>
<td>44.94</td>
<td>27.55</td>
</tr>
</tbody>
</table>

*γ-GT, γ-glutamyltransferase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; sPLA2, secretory phospholipase A2.

*No significant treatment x sex interactions; no significant differences between the control and test groups with baseline value as covariate.
of progression of coronary artery disease (Ascherio et al. 1999; Mensink et al. 2003).

Generally, the differentiation of the physiological effects between individual trans isomers (trans-9-, trans-11-18:1, etc) or between different trans classes (for example, trans-16:1, trans-18:1 and trans-18:2 acids) has rarely been reported in the scientific literature (Mensink et al. 2003). In fact, recent studies demonstrated that higher levels of trans-18:2 and lower levels of trans-18:1 in erythrocyte membranes and plasma lipids are associated with higher risks of fatal IHD and sudden heart death (Lemaitre et al. 2002; Kew et al. 2003). In contrast, CLA, especially the c9,t11 isomer, appear to possess anti-inflammatory and anti-atherogenic properties (Kritchevsky et al. 2004; Jaudszus et al. 2005; Ringseis et al. 2006).

During the present study the supplemented trans isomers incorporated into the lipids of PBMC were increased. In addition, the c9,t11-CLA proportion of PBMC lipids was significantly increased as expected. The source of the increase of c9,t11-CLA concentration was most probably from Δ9-desaturation by SCD with trans-11-18:1 as the precursor. On the contrary, trans-12-18:1 was not converted to c9-9, trans-12-18:2. The quantity of trans-12-18:1 incorporated into the PBMC lipids was approximately 2-fold higher than that of trans-11-18:1.

In general, changes to the types and quantities of dietary fats consumed could influence the production of various cyto- kines and immune cell function in man (Kew et al. 2003). The proportion of trans-fatty acids of erythrocyte membranes has been associated with the increase of primary cardiac arrest (Lemaitre et al. 2002) and the increased concentration of biomarkers of systemic inflammation (TNFα, CRP) in patients with heart diseases (Mozaffarian et al. 2004b). However, there was no evidence of a relationship between trans-fatty acid concentrations in adipose tissue and sudden cardiac death (Roberts et al. 1995). In the present study, the trans-11- and trans-12-18:1 supplementation had no observable effect on the immune cell function and inflammation biomarkers. The determined concentrations of several cytokines, adiponectin and N metabolites did not correlate with the changes of the fatty acid profiles of PBMC (data not shown).

It is possible that the increased c9,t11-CLA could compensate for the effects of the incorporated trans isomers – if they have any effects – whereby the presently synthesised concentrations of c9,t11-CLA (about 0.7 g/d, trans-11 conversion rate 25%) are lower than in previous supplementation studies (24–34 g/d; Tricon et al. 2004; Risérus et al. 2004). However, despite a c9,t11-CLA-rich diet (24 g/d) and a diet naturally enriched with c9,t11-CLA (14 g) and trans-11-18:1 (47 g) in the studies of Burdge et al. (2004), (2005), the c9,t11-CLA content of PBMC lipids did not exceed 0.22 and 0.27% FAME, respectively. The intake of 0.6 g c9,t11-CLA/d (Burdge et al. 2004) compared with endogenously synthesised amounts of c9,t11-CLA (0.7 g/d) during the present study (about 0.7 g/d) showed with similar baseline values (0.08% FAME) slightly lower c9,t11-CLA incorporation into lipids of PBMC than of the endogenously synthesised c9,t11-CLA (0.12 v. 0.16% FAME). In addition, the content of CLA in cellular lipids is dependent on their dietary intake but in general it is not proportional to the CLA intake. The CLA incorporation into cellular lipids is relatively low which can cause the inconclusive and variable effects of CLA supplementation in human subjects (Calder, 2002).

It has been well documented that the composition of cell membranes influences the form and function of these membranes and, thus, potentially affects human health (Han et al. 2002). The supplemented trans-18:1 isomers were readily incorporated into lipids of PBMC and can potentially affect cell membrane functions, and transport and signalling pathways (Katz, 2002).

Trans-fatty acids could also modulate fatty acid metabolism and, possibly, inflammatory responses of adipocytes (Mozaffarian et al. 2006). Adipose tissue acts as an endocrine organ and synthesises adipokines which are suspected of playing a role in inflammation (Nakanishi et al. 2005). Generally, leptin is secreted at concentrations which are proportional to the amount of stored lipids in the human body, and this tendency was observed in the present study. Adiponectin is related to CVD and the metabolic syndrome (Kumada et al. 2003). No changes of leptin and adiponectin concentrations after the trans-11- and trans-12-18:1 supplementation were shown during the present study (Table 5). It is known that leptin is involved in the regulation of SCD which is responsible for the conversion of trans-11-18:1 to c9,t11-CLA. Leptin suppressed the expression and activity of SCD in mice (Cohen & Friedman, 2004). However, in the present study, the concentration of leptin was not associated to the activity of SCD. The SCD activity was estimated by desaturation indices of serum fatty acids (cis-9-18:1:18:0 and cis-9:16:1; Santora et al. 2000).

Trans-fatty acids (for example, trans-11- and trans-12-18:1) as well as CLA are suspected of inducing oxidative stress (8-iso-PGF2α, an isoprostane biomarker of oxidative stress; Turpeinen et al. 2002; Risérus et al. 2002). In contrast, the biomarker of oxidative stress was not affected in a recent study with the supplementation of 3.6 g trans-11-18:1/d over 5 weeks (Tholstrup et al. 2006). Nakanishi et al. (2005) stated that the plasma adiponectin and leptin concentrations were associated with oxidative stress levels. After the application of intervention treatments in the present study, the urinary 8-iso-PGF2α concentrations were observed at higher levels in the test group than those levels observed in the control group (Kuht et al. 2006b). However, no correlation between urinary 8-iso-PGF2α concentrations to leptin and adiponectin concentrations as well as to trans-11-18:1, trans-12-18:1 and CLA of cellular lipids was found (data not shown).

Trans-fatty acids can also modulate monocyte and macrophage activity as manifested by increased production of cytokines (Han et al. 2002). The concentrations of TNFα, IL-1β, IL-6 as well as CRP were considerably increased during the development and progression of inflammation and were reported to be involved in the development of atherosclerotic lesions in man. CRP is increasingly acknowledged as an independent risk factor for CVD and metabolic syndrome (Ridker, 2003).

Recent studies showed that changes in quantity of intake of trans-fatty acids were positively related to changes in plasma IL-6, TNFα (Han et al. 2002) and CRP concentrations (Baer et al. 2004; Mozaffarian et al. 2004a; Lopez-Garcia et al. 2005). In addition, the serum IL-6 concentration was strongly associated with PBMC phospholipid concentrations (Kew et al. 2005). Tholstrup et al. (2006) reported in a butter supplementation
study with healthy men (normal BMI) that both trans-11-18:1 and c9,t11-CLA concentrations increased in plasma, but the plasma CRP concentrations were unchanged. In contrast, in a study of CLA supplementation in human subjects, especially with trans-10, cis-12-CLA, plasma CRP concentrations were increased (Risérus et al. 2002). In the present study, no significant differences in the plasma concentrations of any IL, TNFα and CRP were observed during the trans-11- and trans-12-18:1 intervention. Nevertheless, it is important to note that the plasma cytokine concentration represents the general overall level of the complete body (dilution effects) and not the concentration at the endothelium. Furthermore, the method of CRP concentration determination was fairly insensitive, possessing a detection limit of 3 mg/l. However, at present little is known about the relevance of low concentrations of CRP (0.3 to 1.5 mg/l) in apparently healthy subjects. In a recent study the correlation of plasma PGI2 and TXB2 concentrations which might result from hydrogenated vegetable oils compared with a diet containing SFA (both about 9% energy intake; Turpeinen et al. 1998). In addition, in a rat study, a diet rich in trans-11-18:1 from hydrogenated vegetable oils could affect immune-relevant cells, for example, decrease of lymphocyte proliferation and/or activation, (Thies et al. 2001) and oxidative burst rate by neutrophils (Varming et al. 1995). A diet high in hydrogenated fats, however, did not affect lymphocyte proliferation (Han et al. 2002). The trans-fatty acid composition of the membranes could influence the activity of monocytes and macrophages and this might be relevant for atherosclerotic processes. At present, little if any research has been published concerning the influence of trans-fatty acid isomers on the phagocytic process in human subjects. In the present study, regardless of the extent of trans-11- and trans-12-18:1 incorporation, no significant effects were observed in the cell migration, ingestion and oxidative burst of active cells.

Some studies have reported an association between the intake of trans-fatty acids and the increased risk of CHD in general. Unfortunately, most of these data are from epidemiological studies (for example, Nurses’ Health Study) which are often inconclusive. At present, it is still unknown whether there are any distinctly different effects from the sources of trans-fatty acids (ruminant or industrial; Weggemans et al. 2004), their isomeric distribution, and their general proportion of individual isomers (trans-9- v. trans-11-18 : 1).

In our opinion, conducting long-term trials to test the effects of trans-fatty acid intake would be unethical considering the suggested adverse effects on serum lipids and inflammation. Therefore, in the present study the supplementation period with the high amount of 6 g trans-fatty acid isomers/d over 6 weeks can be classified as a period of high impact on the immune system.

Both supplemented trans isomers (trans-11- and trans-12-18:1) and the synthesised c9,t11-CLA were incorporated into PBMC lipids at least without influencing biomarker concentrations of inflammation and immune function. The Δ9-desaturation of trans-11-18:1 appears to be the key in differentiating the naturally derived trans-11-18:1 isomer from trans-9-18:1, trans-10-18:1, and as presently shown from the trans-12-18:1.

Nevertheless, due to the observed increase of the biomarker 8-iso-PGF2α and the inconsistent and limited published research concerning the effects of trans-fatty acids in human subjects, it is still highly advisable that a general reduction of daily trans-fatty acid intake is recommended, especially in the US and Canadian populations. Further research is required to investigate the effects of the consumption of individual trans-fatty acid isomers on human health.

Acknowledgements

The present study was supported by The German Research Foundation (DFG), JA 893.

References

Albers R, van der Wielen RP, Brink EJ, Hendriks HFJ, Dorovska-Taran VN & Mohede ICM (2003) Effects of cis-9, trans-11 and trans-11-18:1 in plasma, but the plasma CRP concentrations were unchanged. In contrast, in a study of CLA supplementation in human subjects, especially with trans-10, cis-12-CLA, plasma CRP concentrations were increased (Risérus et al. 2002). In the present study, no significant differences in the plasma concentrations of any IL, TNFα and CRP were observed during the trans-11- and trans-12-18:1 intervention. Nevertheless, it is important to note that the plasma cytokine concentration represents the general overall level of the complete body (dilution effects) and not the concentration at the endothelium. Furthermore, the method of CRP concentration determination was fairly insensitive, possessing a detection limit of 3 mg/l. However, at present little is known about the relevance of low concentrations of CRP (0.3 to 1.5 mg/l) in apparently healthy subjects. In a recent study the correlation of plasma PGI2 and TXB2 concentrations which might result from hydrogenated vegetable oils compared with a diet containing SFA (both about 9% energy intake; Turpeinen et al. 1998). In addition, in a rat study, a diet rich in trans-11-18:1 from hydrogenated vegetable oils could affect immune-relevant cells, for example, decrease of lymphocyte proliferation and/or activation, (Thies et al. 2001) and oxidative burst rate by neutrophils (Varming et al. 1995). A diet high in hydrogenated fats, however, did not affect lymphocyte proliferation (Han et al. 2002). The trans-fatty acid composition of the membranes could influence the activity of monocytes and macrophages and this might be relevant for atherosclerotic processes. At present, little if any research has been published concerning the influence of trans-fatty acid isomers on the phagocytic process in human subjects. In the present study, regardless of the extent of trans-11- and trans-12-18:1 incorporation, no significant effects were observed in the cell migration, ingestion and oxidative burst of active cells.

Some studies have reported an association between the intake of trans-fatty acids and the increased risk of CHD in general. Unfortunately, most of these data are from epidemiological studies (for example, Nurses’ Health Study) which are often inconclusive. At present, it is still unknown whether there are any distinctly different effects from the sources of trans-fatty acids (ruminant or industrial; Weggemans et al. 2004), their isomeric distribution, and their general proportion of individual isomers (trans-9- v. trans-11-18 : 1).

In our opinion, conducting long-term trials to test the effects of trans-fatty acid intake would be unethical considering the suggested adverse effects on serum lipids and inflammation. Therefore, in the present study the supplementation period with the high amount of 6 g trans-fatty acid isomers/d over 6 weeks can be classified as a period of high impact on the immune system.

Both supplemented trans isomers (trans-11- and trans-12-18:1) and the synthesised c9,t11-CLA were incorporated into PBMC lipids at least without influencing biomarker concentrations of inflammation and immune function. The Δ9-desaturation of trans-11-18:1 appears to be the key in differentiating the naturally derived trans-11-18:1 isomer from trans-9-18:1, trans-10-18:1, and as presently shown from the trans-12-18:1.

Nevertheless, due to the observed increase of the biomarker 8-iso-PGF2α and the inconsistent and limited published research concerning the effects of trans-fatty acids in human subjects, it is still highly advisable that a general reduction of daily trans-fatty acid intake is recommended, especially in the US and Canadian populations. Further research is required to investigate the effects of the consumption of individual trans-fatty acid isomers on human health.

Acknowledgements

The present study was supported by The German Research Foundation (DFG), JA 893.

References

Albers R, van der Wielen RP, Brink EJ, Hendriks HFJ, Dorovska-Taran VN & Mohede ICM (2003) Effects of cis-9, trans-11 and trans-11-18:1 in plasma, but the plasma CRP concentrations were unchanged. In contrast, in a study of CLA supplementation in human subjects, especially with trans-10, cis-12-CLA, plasma CRP concentrations were increased (Risérus et al. 2002). In the present study, no significant differences in the plasma concentrations of any IL, TNFα and CRP were observed during the trans-11- and trans-12-18:1 intervention. Nevertheless, it is important to note that the plasma cytokine concentration represents the general overall level of the complete body (dilution effects) and not the concentration at the endothelium. Furthermore, the method of CRP concentration determination was fairly insensitive, possessing a detection limit of 3 mg/l. However, at present little is known about the relevance of low concentrations of CRP (0.3 to 1.5 mg/l) in apparently healthy subjects. In a recent study the correlation of plasma PGI2 and TXB2 concentrations which might result from hydrogenated vegetable oils compared with a diet containing SFA (both about 9% energy intake; Turpeinen et al. 1998). In addition, in a rat study, a diet rich in trans-11-18:1 from hydrogenated vegetable oils could affect immune-relevant cells, for example, decrease of lymphocyte proliferation and/or activation, (Thies et al. 2001) and oxidative burst rate by neutrophils (Varning et al. 1995). A diet high in hydrogenated fats, however, did not affect lymphocyte proliferation (Han et al. 2002). The trans-fatty acid composition of the membranes could influence the activity of monocytes and macrophages and this might be relevant for atherosclerotic processes. At present, little if any research has been published concerning the influence of trans-fatty acid isomers on the phagocytic process in human subjects. In the present study, regardless of the extent of trans-11- and trans-12-18:1 incorporation, no significant effects were observed in the cell migration, ingestion and oxidative burst of active cells.

Some studies have reported an association between the intake of trans-fatty acids and the increased risk of CHD in general. Unfortunately, most of these data are from epidemiological studies (for example, Nurses’ Health Study) which are often inconclusive. At present, it is still unknown whether there are any distinctly different effects from the sources of trans-fatty acids (ruminant or industrial; Weggemans et al. 2004), their isomeric distribution, and their general proportion of individual isomers (trans-9- v. trans-11-18 : 1).

In our opinion, conducting long-term trials to test the effects of trans-fatty acid intake would be unethical considering the suggested adverse effects on serum lipids and inflammation. Therefore, in the present study the supplementation period with the high amount of 6 g trans-fatty acid isomers/d over 6 weeks can be classified as a period of high impact on the immune system.

Both supplemented trans isomers (trans-11- and trans-12-18:1) and the synthesised c9,t11-CLA were incorporated into PBMC lipids at least without influencing biomarker concentrations of inflammation and immune function. The Δ9-desaturation of trans-11-18:1 appears to be the key in differentiating the naturally derived trans-11-18:1 isomer from trans-9-18:1, trans-10-18:1, and as presently shown from the trans-12-18:1.

Nevertheless, due to the observed increase of the biomarker 8-iso-PGF2α and the inconsistent and limited published research concerning the effects of trans-fatty acids in human subjects, it is still highly advisable that a general reduction of daily trans-fatty acid intake is recommended, especially in the US and Canadian populations. Further research is required to investigate the effects of the consumption of individual trans-fatty acid isomers on human health.

Acknowledgements

The present study was supported by The German Research Foundation (DFG), JA 893.

References


