British Journal of Nutrition (2007), **97**, 1196–1205
doi: 10.1017/S0007114507685183

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 Kuhnt[1], Jana Kraft[1], Heinz Vogelsang[2], Klaus Eder[3], Jürgen Kratzsch[4] and Gerhard Jahreis[1]*

[1]*Institute of Nutrition, Friedrich Schiller University, Dornburger Strasse 24, D-07743 Jena, Germany*
[2]*Institute of Clinical Chemistry and Laboratory Diagnostics, Friedrich Schiller University, Jena, Germany*
[3]*Institute of Nutrition, Martin Luther University, Halle/Saale, Germany*
[4]*Institute 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 (6·0 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 (*c*9,*t*11)-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 & Griinari, 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.* 2006*a*).

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: *c*9,*t*11, *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-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; 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.* 2004*a*,*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; *c*9,*t*11 and *trans*-10, *cis*-12) were found in cell and animal studies to have anti-inflammatory activity (suppressing eicosanoid synthesis; for example, prostaglandin E_2 and prostaglandin I_2 (prostacyclin; PGI_2) (Bulgarella *et al.* 2001) and pro-inflammatory cytokines, for example, TNFα and IL-8) (Jaudszus *et al.* 2005; Ringseis *et al.* 2006) and to reduce fatty streak formation (Kritchevsky *et al.* 2004). Furthermore, CLA can alter immune function (for example, cell proliferation (Bassaganya-Riera *et al.* 2003; Hontecillas *et al.* 2003); immunglobulins (Bontempo *et al.* 2004)). However, the majority of human CLA studies reported less consistent responses than those of the animal studies (Kelley *et al.* 2001; Albers *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, PGI_2, activity of phospholipase A_2, and transaminases). In addition, we determined the fatty acid composition and the incorporation of *trans*-11-18 : 1 and *trans*-12-18 : 1 and their Δ9-desaturation products (*c*9,*t*11-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.* 2006*a*). 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.

Table 1. Baseline data of female and male subjects at the beginning of the intervention period

(Mean values and standard deviations)

	Women (*n* 12)		Men (*n* 12)		
	Mean	SD	Mean	SD	*P* for sex*
Age (years)	24	3	25	2	NS
Weight (kg)	54	6	75	5	<0·001
Height (cm)	166	6	185	7	<0·001
BMI (kg/m^2)	19·7	2	22·0	2	0·011
Body fat mass (%)	22·4	4·5	15·8	5·8	0·005
Plasma					
Total cholesterol (mmol/l)	178	48	171	65	NS
LDL-cholesterol:HDL-cholesterol	122	48	159	48	NS
TAG (mmol/l)	1·0	0·3	1·0	0·4	NS

* *t* test; $P \leq 0{\cdot}05$.

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***,

Table 2. Daily dietary intake during the standardised diet of the study
(Mean values and standard deviations)

		Adaptation period				Intervention period				
		Control group		Test group		Control group		Test group		
Daily intake		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Treatment effect*† (P)
Energy (MJ)	Women	8·6	1·9	9·3	1·5	8·7	1·1	8·5	2·3	NS
	Men	11·3	1·1	10·6	1·4	11·4	0·5	10·9	1·3	NS
Carbohydrates (% energy intake)	Women	62·9	4·1	63·2	3·1	60·1	3·4	59·5	4·0	NS
	Men	59·8	1·8	62·9	3·8	58·7	1·2	60·4	4·0	NS
Protein (% energy intake)	Women	12·0	1·2	12·3	0·6	11·9	1·1	12·2	1·1	NS
	Men	12·7	0·2	12·3	0·5	12·4	0·3	12·3	0·5	NS
Fat (% energy intake)	Women	26·9	3·2	26·1	2·7	29·5	2·9	30·0	3·3	NS
	Men	29·0	1·5	27·4	4·1	30·6	0·8	29·1	4·5	NS
Fatty acids (% fat intake)										
SFA	Women	25·4	2·5	26·4	1·8	26·3	2·0	25·5	2·7	NS
	Men	24·8	3·2	25·8	3·0	26·0	3·0	24·9	2·8	NS
cis-MUFA	Women	34·1	1·7	33·1	2·5	33·9	1·7	31·4	2·2	NS
	Men	31·9	0·3	31·8	3·2	32·1	0·9	30·9	2·4	NS
trans-MUFA	Women	0·2	0·0	0·2	0·0	0·1	0·0	9·5	1·8	$<0{\cdot}001$
	Men	0·2	0·0	0·2	0·0	0·2	0·0	7·3	1·1	$<0{\cdot}001$
PUFA	Women	26·5	3·6	26·3	3·0	26·9	3·5	22·1	2·7	$<0{\cdot}05$
	Men	30·0	2·6	29·0	1·8	30·4	4·9	25·2	5·5	$<0{\cdot}001$
TE (mg/g fat)‡	Women	0·20	0·04	0·23	0·04	0·18	0·04	0·21	0·04	NS
	Men	0·23	0·02	0·21	0·03	0·21	0·01	0·20	0·02	NS

TE, tocopherol equivalents.
* No significant treatment × sex interactions.
† Significantly different from the control group with baseline value as covariate.
‡ 1 mg TE = 1 mg α-tocopherol = 10 mg γ-tocopherol.

10 min) to lower the degree of erythrocyte contamination. Cell count was determined using a Neubauer haematocytometer 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.* 2006*a*). Briefly, total lipids of PBMC (at least 20×10^6) 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 from C4 to C25 carbon length including CLA using a fused silica capillary column (DB-225 ms, 60 m × 0·25 mm internal diameter with 0·25 μm film thickness; J&W Scientific, Folsom, CA, USA). The second GC method separated the *cis* and *trans* isomers of 18 : 1 using a fused silica capillary column (CP-select, 200 m × 0·25 mm internal diameter with 0·25 μm film thickness; Varian, Middelburg, The Netherlands). For both procedures the injector and detector temperatures were maintained at 260 and 270°C, respectively. H_2 was used as the carrier gas at 2·22 ml/min. The first GC method was as follows: the initial oven temperature was maintained at 70°C for 2 min, then increased at 10°C/min to 180°C, then increased at 2°C/min to 220°C and held for 5 min and finally, it was increased at 2°C/min to 230°C and held for 15 min. The second GC method required isothermal conditions at 181°C. The 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.

Immunophenotyping

The two-colour immunophenotyping was conducted by flow cytometry in a flow cytometer FACScan™ employing simultSET™ 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 Migratest® 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 chemoattractant 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 O_2-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-8, 1β, 6, 10, 12p70) 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-8, 1β, 6, 10 and 12p70 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 A_2 activity

The effects of the *trans*-11- and *trans*-12-18 : 1 supplementation on secretory phospholipase A_2 ($sPLA_2$) activity in plasma were assessed using an $sPLA_2$ assay kit (Cayman Chemical, Ann Arbor, MI, USA). PGI_2, an endothelial prostglandin, is quickly hydrated to its more stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). The plasma 6-keto-$PGF_{1\alpha}$ metabolite concentrations were utilised to estimate PGI_2 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-$PGF_{1\alpha}$ 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 immunoassay 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 $\leq 0{\cdot}05$ 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). *c*9,*t*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 : 6*n*-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)

	Adaptation period (day 0)				Intervention period (day 42)				
	Control group (*n* 12)		Test group (*n* 12)		Control group (*n* 12)		Test group (*n* 12)		
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Treatment effect*† (P)
16 : 0	19·84	1·64	19·28	3·43	20·06	2·13	19·79	2·62	NS
cis-9-16 : 1	0·86	0·07	0·79	0·14	0·81	0·09	0·85	0·14	NS
18 : 0	22·34	1·22	21·40	2·74	22·16	1·43	20·91	2·34	NS
cis-9-18 : 1	15·32	0·83	14·80	1·61	15·53	1·75	16·13	1·71	NS
trans-11-18 : 1	0·05	0·03	0·06	0·05	0·05	0·04	0·45	0·06	<0·001
trans-12-18 : 1	0·00	0·00	0·00	0·00	0·00	0·00	0·83	0·12	<0·001
cis-9, *cis*-12-18 : 2	6·99	0·43	6·76	0·91	6·97	0·76	6·61	0·56	NS
cis-9, *trans*-12-18 : 2	0·07	0·00	0·08	0·01	0·07	0·00	0·07	0·01	NS
cis-9, *trans*-11-18 : 2‡	0·08	0·01	0·08	0·01	0·07	0·01	0·16	0·03	<0·001
20 : 4*n*-6	11·67	1·90	11·19	1·57	11·79	2·82	11·11	2·85	NS
20 : 5*n*-3	0·12	0·03	0·18	0·05	0·12	0·04	0·16	0·09	NS
22 : 6*n*-3	0·53	0·32	0·49	0·27	0·54	0·36	0·33	0·12	0·003

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

group 6812 (SD 4156); test group 6459 (SD 4588)). The percentage of phagocytic granulocytes in both treatment groups was unaffected by the intervention treatments (control group 97·4 (SD 1·7) %; test group 97·8 (SD 1·9) %). In addition, no significant differences for both treatment groups were observed between the percentage of granulocytes with oxidative burst (control group 94·1 (SD 4·3) %; test group 95·8 (SD 4·2) %) and their individual cell activity.

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

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)

	Adaptation period (day 0)				Intervention period (day 42)*			
	Test group (*n* 12)		Control group (*n* 12)		Test group (*n* 12)		Control group (*n* 12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total leucocytes (CD45-carrying cells) (%)								
Lymphocytes	38·6	8·8	42·0	9·9	39·3	7·1	39·1	10·1
Monocytes	6·7	1·0	6·0	1·4	5·8	1·4	6·2	0·9
Granulocytes	54·7	8·7	51·7	10·1	55·2	7·2	54·9	9·5
Total lymphocytes (%)								
$CD3^+$	68·8	7·1	66·8	8·4	66·3	5·2	64·6	7·5
$CD3^+/CD4^+$	40·3	4·8	42·1	9·0	39·3	3·0	40·0	8·5
$CD3^+/CD8^+$	23·8	5·7	23·1	4·5	24·4	4·8	22·5	4·8
$CD4^+CD8^+$	1·9	0·7	1·9	0·6	1·7	0·4	1·9	0·7
$CD19^+$	11·2	3·2	11·1	3·1	11·2	3·7	10·5	2·8
$CD3^-CD16^+/CD56^+$	16·1	6·4	17·6	9·2	17·1	6·8	20·3	9·3
$CD3^+$HLA-DR^+	4·4	1·1	3·7	1·3	5·1	1·5	4·1	1·9
CD57	11·4	4·1	9·3	5·8	11·6	6·7	10·4	5·4
$CD8^+$CD57	5·1	2·1	4·4	2·9	5·8	2·2	5·6	2·7
CD25	19·8	3·3	18·4	4·3	21·6	4·7	18·6	4·9
$CD4^+CD25^+$	13·8	3·6	12·8	3·7	15·0	2·9	13·1	4·1
CD54	59·4	8·7	59·8	10·7	56·4	10·9	60·3	10·0
$CD4^+CD54^+$	12·3	3·5	10·8	4·1	11·4	3·1	11·3	3·9
CD130	41·7	8·6	42·6	9·8	37·9	10·3	36·4	10·8
$CD4^+CD130^+$	26·1	5·3	28·7	7·7	25·3	6·0	25·2	7·8

CD, cluster of differentiation.
* No significant treatment × sex interactions; no significant differences between the control and test groups with baseline value as covariate.

and intervention periods (data not shown; $P \leq 0{\cdot}017$). Furthermore, the concentration of urea correlated with uric acid (control group r 0·614, $P=0{\cdot}034$; test group r 0·399, $P=0{\cdot}199$) and creatinine (control group r 0·586, $P=0{\cdot}045$; test group r 0·709, $P=0{\cdot}010$). 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; $P \leq 0{\cdot}05$).

Plasma concentrations of cytokines, adipokines, and 6-keto-prostaglandin $F_{1\alpha}$

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, $P=0{\cdot}001$; test group r 0·634, $P=0{\cdot}027$) and IL-8 and TNFα (intervention period: control group r 0·562, $P=0{\cdot}057$; test group r 0·763, $P=0{\cdot}004$).

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 10·5 (SD 9·4) ng/ml *v.* male 2·3 (SD 2·1) ng/ml; $P=0{\cdot}007$). In contrast, females' adiponectin concentrations were lower than that of their male counterparts (female 11·3 (SD 4·6) ng/ml *v.* male 14·8 (SD 4·5) ng/ml; $P=0{\cdot}073$). 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, $P<0{\cdot}001$; female r 0·774, $P=0{\cdot}005$; intervention period: male r 0·786, $P=0{\cdot}002$; female r 0·779, $P=0{\cdot}005$). 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 $sPLA_2$ and the plasma concentration of 6-keto-$PGF_{1\alpha}$ 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.* 2004*a*). 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 5. The effects of the dietary supplementation of *trans*-11- and *trans*-12-18 : 1 isomers (6 g/d; 1 : 1) on the concentrations of plasma biomarkers

(Mean values and standard deviations)

	Adaptation period (day 0)				Intervention period (day 42)*			
	Control group (*n* 12)		Test group (*n* 12)		Control group (*n* 12)		Test group (*n* 12)	
Plasma biomarker	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bilirubin (μmol/l)	6·6	3·2	7·1	3·4	7·9	4·5	10·0	5·3
Urea (mmol/l)	2·0	0·6	2·3	0·8	2·5	0·9	2·2	0·9
Uric acid (μmol/l)	154	54	162	50	195	79	239	71
Creatinine (μmol/l)	41	15	43	13	51	26	62	19
γ-GT activity (μmol/s)	0·32	0·07	0·32	0·11	0·26	0·09	0·26	0·11
ALAT activity (μmol/l per s)	0·31	0·16	0·26	0·13	0·19	0·12	0·16	0·05
ASAT activity (μmol/l per s)	0·41	0·14	0·34	0·13	0·28	0·10	0·23	0·07
TNFα (pg/ml)	3·93	1·88	2·86	1·47	3·15	1·85	3·05	1·45
IL-1β (pg/ml)	38·11	35·42	46·37	47·91	22·61	19·85	33·91	31·43
IL-6 (pg/ml)	1·83	1·45	2·28	1·98	2·05	1·75	2·32	1·86
IL-8 (pg/ml)	37·71	32·36	29·56	21·45	43·62	22·75	40·62	24·61
IL-10 (pg/ml)	7·73	4·64	7·28	5·18	6·53	2·44	6·69	3·27
IL-12-p70 (pg/ml)	12·87	8·56	13·62	9·61	9·36	7·28	13·53	9·86
Leptin (ng/ml)	4·82	3·86	5·77	4·13	4·89	4·02	5·21	4·53
Adiponectin (mg/ml)	7·26	2·26	6·69	2·49	6·33	2·34	6·69	2·67
$sPLA_2$ activity (pmol/min per l)	0·25	0·07	0·26	0·09	0·19	0·08	0·19	0·06
6-Keto-$PGF_{1\alpha}$ (pg/ml)	44·94	27·55	44·88	31·41	36·85	20·87	39·02	28·31

γ-GT, γ-glutamyltransferase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; $sPLA_2$, secretory phospholipase A_2.
* No significant treatment × 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.* 2006; Kew *et al.* 2003). In contrast, CLA, especially the *c*9,*t*11 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 *c*9,*t*11-CLA proportion of PBMC lipids was significantly increased as expected. The source of the increase of *c*9,*t*11-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 *cis*-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 cytokines 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.* 2004*b*). 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, adipokines 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 *c*9,*t*11-CLA could compensate for the effects of the incorporated *trans* isomers – if they have any effects – whereby the presently synthesised concentrations of *c*9,*t*11-CLA (about 0·7 g/d, *trans*-11 conversion rate 25 %) are lower than in previous supplementation studies (2·4–3·0 g/d; Tricon *et al.* 2004; Risérus *et al.* 2004). However, despite a *c*9,*t*11-CLA-rich diet (2·4 g/d) and a diet naturally enriched with *c*9,*t*11-CLA (1·4 g) and *trans*-11-18 : 1 (4·7 g) in the studies of Burdge *et al.* (2004), (2005), the *c*9,*t*11-CLA content of PBMC lipids did not exceed 0·22 and 0·27 % FAME, respectively. The intake of 0·6 g *c*9,*t*11-CLA/d (Burdge *et al.* 2004) compared with endogenously synthesised amounts of *c*9,*t*11-CLA (0·7 g/d) during the present study (about 0·7 g/d) showed with similar baseline values (0·08 % FAME) slightly lower *c*9,*t*11-CLA incorporation into lipids of PBMC than of the endogenously synthesised *c*9,*t*11-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 *c*9,*t*11-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 : 1/16 : 0; 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-$PGF_{2\alpha}$, 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-$PGF_{2\alpha}$ concentrations were observed at higher levels in the test group than those levels observed in the control group (Kuhnt *et al.* 2006*b*). However, no correlation between urinary 8-iso-$PGF_{2\alpha}$ 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.* 2004*a*; Lopez-Garcia *et al.* 2005). In addition, the serum IL-6 concentration was strongly associated with PBMC phospholipid concentrations (Kew *et al.* 2003). Tholstrup *et al.* (2006) reported in a butter supplementation

study with healthy men (normal BMI) that both *trans*-11-18 : 1 and *c*9,*t*11-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 CRP and CHD was assessed as relatively moderate (Danesh *et al.* 2004). Thus, it is arguable whether the correlation of *trans*-fatty acid intake and plasma CRP is evident. Moreover, the pro-inflammatory effects of dietary *trans*-fatty acids were observed in women with increased BMI (Mozaffarian *et al.* 2004*a*). This observation suggests that the *trans*-fatty acid intake could be related to effects on and responses of adipose tissue or stored fat. In subjects with normal BMI no significant relationship was observed in the present study and in the studies of Mozaffarian *et al.* (2004*a*) and Tholstrup *et al.* (2006).

Trans-fatty acids could change lipoprotein metabolism (Mensink *et al.* 2003). However, in the present study with normocholesterolaemic subjects the total cholesterol:HDL-cholesterol ratio and the LDL-cholesterol:HDL-cholesterol ratio were not affected by the study treatments. In the study with 3·6 g *trans*-11-18 : 1/d over 5 weeks no changes of serum lipids were observed as well (Tholstrup *et al.* 2006).

One cell study confirmed that CLA can directly reduce the production of 6-keto-$PGF_{1\alpha}$ in human vein endothelial cells (Torres-Duarte *et al.* 2003). *Trans*-fatty acids could also affect thrombogenesis due to their influence on the eicosanoid synthesis. However, in a human intervention study the platelet activation and endothelial PGI_2 production was unchanged during a 5-week diet supplemented with *trans*-fatty acids 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*-18 : 1 fatty acids decreased the arachidonic acid of aorta and platelet phospholipids, yet no observable effects were detected in the plasma PGI_2 and TXB2 concentrations which might result from an adequate supply of linoleic acid (Mahfouz & Kummerow, 1999). The $sPLA_2$ could be additionally a relevant biomarker for atherogenesis. However, in the present study the $sPLA_2$ activity and the plasma concentration of 6-keto-$PGF_{1\alpha}$ were not influenced by the *trans*-11- and *trans*-12-18 : 1 supplement treatment. No correlation with *trans*-11- and *trans*-12-18 : 1, or *c*9,*t*11-CLA proportions of PBMC lipids of both treatment groups were observed (data not shown).

Trans-fatty acid intake has been reported to increase the concentrations of biomarkers related to endothelial dysfunction (Lopez-Garcia *et al.* 2005). Diets rich in CLA (*c*9,*t*11 and *trans*-10, *cis*-12) and *trans*-11-18 : 1 did not affect blood pressure and arterial elasticity in healthy men (Raff *et al.* 2006). The present study showed no effects on ICAM-1 on total leucocytes. Unfortunately, we did not investigate other variables that represent endothelial function (for example, E-selectin, blood pressure).

Dietary fatty acids 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 *c*9,*t*11-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-$PGF_{2\alpha}$ 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-10, *cis*-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. *Eur J Clin Nutr* **57**, 595–603.
Aro A, Kosmeijeir-Schuil T, van den Bovenkamp P, Hulshof P, Zock P & Katan MB (1998) Analysis of C18 : 1 *cis* and *trans* fatty acid isomers by the combination of gas-liquid chromatography of 4,4-dimethyloxazoline derivatives and methyl esters. *J Am Oil Chem Soc* **75**, 977–985.
Ascherio A, Katan MB, Zock PL, Stampfer MJ & Willett WC (1999) *Trans* fatty acids and coronary heart disease. *N Engl J Med* **340**, 1994–1998.
Baer DJ, Judd JT, Clevidence BA & Tracy RP (2004) Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr* **79**, 969–973.
Bassaganya-Riera J, Pogranichniy RM, Jobgen SC, Halbur PG, Yoon KJ, O'Shea M, Mohede I & Hontecillas R (2003) Conjugated linoleic acid ameliorates viral infectivity in a pig model of virally induced immunosuppression. *J Nutr* **133**, 3204–3214.
Bauman DE & Griinari JM (2003) Nutritional regulation of milk fat synthesis. *Annu Rev Nutr* **23**, 203–227.
Bontempo V, Sciannimanico D, Pastorelli G, Rossi R, Rosi F & Corino C (2004) Dietary conjugated linoleic acid positively affects immunologic variables in lactating sows and piglets. *J Nutr* **134**, 817–824.
Bray GA, Lovejoy JC, Smith SR, DeLany JP, Lefevre M, Hwang D, Ryan DH & York DA (2002) The influence of different fats and fatty acids on obesity, insulin resistance and inflammation. *J Nutr* **132**, 2488–2491.
Bulgarella JA, Patton D & Bull AW (2001) Modulation of prostaglandin H synthase activity by conjugated linoleic acid (CLA) and specific CLA isomers. *Lipids* **36**, 407–412.
Burdge GC, Lupoli B, Russell JJ, *et al.* (2004) Incorporation of *cis*-9,*trans*-11 or *trans*-10,*cis*-12 conjugated linoleic acid into plasma and cellular lipids in healthy men. *J Lipid Res* **45**, 736–741.
Burdge GC, Tricon S, Morgan R, *et al.* (2005) Incorporation of *cis*-9, *trans*-11 conjugated linoleic acid and vaccenic acid (*trans*-11 18 : 1) into plasma and leucocyte lipids in healthy men consuming dairy products naturally enriched in these fatty acids. *Br J Nutr* **94**, 237–243.
Calder PC (2002) Conjugated linoleic acid in humans - reasons to be cheerful? *Curr Opin Clin Nutr Metab Care* **5**, 123–126.
Cohen P & Friedman JM (2004) Leptin and the control of metabolism: role for stearoyl-CoA desaturase-1 (SCD-1). *J Nutr* **134**, S2455–S2463.
Craig-Schmidt MC (1998) World wide consumption of *trans* fatty acids. In *Trans Fatty Acids in Human Nutrition*, pp. 59–114 [JL Sebedio and WW Christie, editors]. Dundee, UK: The Oily Press.
Danesh J, Wheeler J, Hirschfield G, Eda S, Eiriksdottir G, Rumley A, Lowe G, Pepys M & Gudnason V (2004) C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* **350**, 1387–1397.
Delmonte P, Roach JAG, Mossoba MM, Losi G & Yurawecz MP (2004) Synthesis, isolation, and GC analysis of all the 6,8- to 13,15-*cis*/*trans* conjugated linoleic acid isomers. *Lipids* **39**, 185–191.
Food and Drug Administration (2003) Food labeling: *trans* fatty acids in nutrition labelling, nutrient content claims, and health claims. *Fed Regist* **68**, 41434–41506.
Food and Drug Administration, (2006). *Trans* Fatty Acids section. US FDA/CFSAN Food Labeling Specific Topics and Categories (updated January 2006). Accessed 12 January 2007, available at http://www.cfsan.fda.gov/~dms/lab-cat.html#transfat.
Fremann D, Linseisen J & Wolfram G (2002) Dietary conjugated linoleic acid (CLA) intake assessment and possible biomarkers of CLA intake in young women. *Public Health Nutr* **5**, 73–80.
Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ & Meydani SN (2002) Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *J Lipid Res* **43**, 445–452.
Hontecillas R, Wannemeulher MJ, Zimmerman DR, Hutto DL, Wilson JH, Ahn DU & Bassaganya-Riera J (2003) Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid. *J Nutr* **132**, 2019–2027.
Jahreis G & Kraft J (2002) Sources of conjugated linoleic acid in the human diet. *Lipid Tech* **14**, 29–32.
Jaudszus A, Foerster M, Kroegel C, Wolf I & Jahreis G (2005) *Cis*-9,-*trans*-11-CLA exerts anti-inflammatory effects in human bronchial epithelial cells and eosinophils: comparison to *trans*-10,*cis*-12-CLA and to linoleic acid. *Biochim Biophys Acta* **1737**, 111–118.
Katz AM (2002) *Trans*-fatty acids and sudden cardiac death. *Circulation* **105**, 669–671.
Kelley DS, Simon VA, Taylor PC, Rudolph IL, Benito P, Nelson GJ & Mackey BE (2001) Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells, but did not alter their function. *Lipids* **36**, 669–674.
Kew S, Banerjee T, Minihane AM, Finnegan YE, Williams CM & Calder PC (2003) Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. *Am J Clin Nutr* **77**, 1278–1286.
Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM, Yaqoob P (2004) Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell. *Am J Clin Nutr* **79**, 674–681.
Kraft J, Collomb M, Moeckel P, Sieber R & Jahreis G (2003) Differences in CLA isomer distribution of cow's milk lipids. *Lipids* **38**, 657–664.
Kraft J, Hanske L, Moeckel P, Zimmermann S, Haertl A, Kramer JKG & Jahreis G (2006) The conversion efficiency of *trans*-11 and *trans*-12-18 : 1 by Δ9-desaturation differs in rats. *J Nutr* **136**, 1209–1214.
Kratzsch J, Berthold A, Lammert A, Reuter W, Keller E & Kiess W (2002) A rapid, quantitative immunofunctional assay for measuring human leptin. *Horm Res* **57**, 127–132.
Kritchevsky D, Tepper SA, Wright S, Czarnecki SK, Wilson TA & Nicolosi RJ (2004) Conjugated linoleic acid isomer effects in atherosclerosis: growth and regression of lesions. *Lipids* **39**, 611–616.
Kuhnt K, Kraft J, Moeckel P & Jahreis G (2006*a*) *Trans*-11-18 : 1 is effectively Δ9-desaturated compared with *trans*-12-18 : 1 in humans. *Br J Nutr* **95**, 752–761.
Kuhnt K, Wagner A, Kraft J, Basu S & Jahreis G (2006*b*) Dietary supplementation with *trans*11 C18 : 1 and *trans*12 C18 : 1 and oxidative stress in humans. *Am J Clin Nutr* **84**, 981–988.
Kumada M, Kihara S, Sumitsuji S, *et al.* (2003) Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* **23**, 85–89.
Lefevre M, Lovejoy JC, Smith SR, DeLany JP, Champagne C, Most MM, Denkins Y, de Jonge L, Rood J & Bray GA (2005) Comparison of the acute response to meals enriched with *cis*- or *trans*-fatty acids on glucose and lipids in overweight individuals with differing FABP2 genotypes. *Metabolism* **54**, 1652–1658.
Lemaitre RN, King IB, Mozaffarian D, Kuller LH, Tracy RP, Sotoodehnia N & Siscovick DS (2006) Plasma phospholipid *trans*-fatty acids and fatal ischemic heart disease in older adults. The Cardiovascular Health Study. *Circulation* **114**, 209–215.
Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copass MK, Cobb LA & Siscovick DS (2002) Cell membrane *trans*-fatty acids and the risk of primary cardiac arrest. *Circulation* **105**, 697–701.
Libby P (2002) Inflammation in atherosclerosis. *Nature* **420**, 868–874.

Lopez-Garcia E, Schulze MB, Meigs JB, Manson JAE, Rifai N, Stampfer MJ, Willett WC & Hu FB (2005) Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* **135**, 562–566.

Mahfouz MM & Kummerow FA (1999) Hydrogenated fat high in *trans* monoenes with an adequate level of linoleic acid has no effect on prostaglandin synthesis in rats. *J Nutr* **129**, 15–24.

Mensink RP, Zock PL, Kester AD & Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* **77**, 1146–1155.

Mosley EE, Shafii B, Moate PJ & McGuire MA (2006) *Cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. *J Nutr* **136**, 570–575.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ & Willett WC (2006) Medical progress – *trans* fatty acids and cardiovascular disease. *N Engl J Med* **354**, 1601–1613.

Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC & Rimm EB (2004*a*) Dietary intake of *trans* fatty acids and systemic inflammation in women. *Am J Clin Nutr* **79**, 606–612.

Mozaffarian D, Rimm EB, King IB, Lawler RL, McDonald GB & Levy WC (2004*b*) *Trans* fatty acids and systemic inflammation in heart failure. *Am J Clin Nutr* **80**, 1521–1525.

Nakanishi S, Yamane K, Kamei N, Nojima H, Okubo M & Kohno N (2005) A protective effect of adiponectin against oxidative stress in Japanese Americans: the association between adiponectin or leptin and urinary isoprostane. *Metabolism* **54**, 194–199.

Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC (2005) Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. *Am J Epidemiol* **161**, 672–679.

Raff M, Tholstrup T, Sejrsen K, Straarup EM & Wiinberg N (2006) Diets rich in conjugated linoleic acid and vaccenic acid have no effect on blood pressure and isobaric arterial elasticity in healthy young men. *J Nutr* **136**, 992–997.

Ridker PM (2003) Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* **107**, 363–369.

Ringseis R, Mueller A, Herter C, Gahler S, Steinhart H & Eder K (2006) CLA isomers inhibit TNF α-induced eicosanoid release from human vascular smooth muscle cells *via* a PPAR-γ ligand-like action. *Biochim Biophys Acta* **1760**, 290–300.

Risérus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J & Vessby B (2002) Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein – a potential link to fatty acid-induced insulin resistance. *Circulation* **106**, 1925–1929.

Risérus U, Vessby B, Arnlov J & Basu S (2004) Effects of *cis*-9,*trans*-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men. *Am J Clin Nutr* **80**, 279–283.

Roberts TL, Wood DA, Riemersma RA, Gallagher PJ & Lampe FC (1995) *Trans* isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. *Lancet* **345**, 278–282.

Santora JE, Palmquist DL & Roehrig KL (2000) *Trans*-vaccenic acid is desaturated to conjugated linoleic acid in mice. *J Nutr* **130**, 208–215.

Thies F, Nebe-von-Garon G, Powell JR, Yaqoob P, Newsholme EA & Calder PC (2001) Dietary supplementation with γ-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *J Nutr* **131**, 1918–1927.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K & Straarup E (2006) Effects of butter high in ruminant *trans* on lipoproteins, fatty acid incorporation in lipid classes, C-reactive protein, oxidative stress, hemostatic variables and insulin in healthy, young men. *Am J Clin Nutr* **83**, 237–243.

Torres-Duarte AP & Vander Hoek JY (2003) Conjugated linoleic acid exhibits stimulatory and inhibitory effects on prostanoid production in human endothelial cells and platelets. *Biochim Biophys Acta Molec Cell Res* **1640**, 69–76.

Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC & Yaqoob P (2004) Effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on immune cell function in healthy humans. *Am J Clin Nutr* **80**, 1626–1633.

Turpeinen AM, Mutanen MAA, Salminen I, Basu S, Palmquist DL & Griinari JM (2002) Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am J Clin Nutr* **76**, 504–510.

Turpeinen AM, Wuebert J, Aro A, Lorenz R & Mutanen M (1998) Similar effects of diets rich in stearic acid or *trans*-fatty acids on platelet function and endothelial prostacyclin production in humans. *Arterioscler Thromb Vasc Biol* **18**, 316–322.

van de Vijver LPL, Kardinaal AFM, Couet C, *et al.* (2000) Association between *trans* fatty acid intake and cardiovascular risk factors in Europe: the TRANSFAIR study. *Eur J Clin Nutr* **54**, 126–135.

Varming K, Schmidt EB, Svaneborg N, Moller JM, Lervang HH, Grunnet N, Jersild C & Dyerberg J (1995) The effect of *n*-3 fatty-acids on neutrophil chemiluminescence. *Scand J Clin Lab Invest* **55**, 47–52.

Weggemans RM, Rudrum M & Trautwein EA (2004) Intake of ruminant versus industrial trans fatty acids and risk of coronary heart disease: what is the evidence? *Eur J Lipid Sci Technol* **106**, 390–397.

Wolff RL (1995) Content and distribution of *trans*-18 : 1 acids in ruminant milk and meat fats. Their importance in European diets and their effect on human milk. *J Am Oil Chem Soc* **72**, 259–272.