The Δ9-desaturation of dietary *trans* octadecenoic acids (*trans*11 and *trans*12 18:1) and the clinical relevance in humans

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PREAMBLE

LIST OF ABBREVIATIONS

Σ; total

15-kd-$PGF_{2\alpha}$; 15-keto-13,14-dihydro-$PGF_{2\alpha}$

18:1; octadecenoic acid

8-iso-$PGF_{2\alpha}$; 8-iso-prostaglandin $F_{2\alpha}$

8-oxodG; 7,8-dihydro-8-oxo-2´-deoxy-guanosine

AA; arachidonic acid

ACOX; acyl-CoA oxidase

ALAT; alanine-aminotransferase

ASAT; aspartate-aminotransferase

BCL2; B-cell CLL/lymphoma 2

BIA; bioimpedance analysis

BMI; body mass index

C; cholesterol

c; *cis*

*c*9,*t*11 CLA; *cis*9,*trans*11 18:2

CD; cluster of differentiation

CLA; conjugated linoleic acids

COX; cyclooxygenase

Cr; creatinine

CRP; C-reactive protein

CYP4A11; cytochrome p450 monooxygenase

d; day

DNA; deoxyribonucleic acid

EFSA; European Food Safety Authority

EU; European Union

FABP; fatty acid binding protein

FAME; fatty acid methyl esters

FAT; fatty acid translocase

FATP; fatty acid transport protein

FDA; Food and Drug Administration

GC; gas chromatograph

GLUT; glucose transporter

HDL; high density lipoprotein

HPLC; high performance liquid chromatography

ICAM; intercellular adhesion molecule

Ig; immunglobulin

IL; interleukin

LDL; low density lipoprotein

LOX; lipoxygenase

LPL; lipoprotein lipase

m; men

ME; malic enzyme

MUFA; mono unsaturated fatty acids

NAD; nicotinamide adenine dinucleotide

NK; natural killer cells

NS; not significant

PBE; peroxisomal bifunctional enzyme

PBS; phosphate buffered saline

PG; prostaglandin

PGI_2; prostacyclin

PHVO; partially hydrogenated vegetable oils

PL; phospholipids

PBMC; peripheral blood mononuclear cells

PPAR; peroxisome proliferator-activated receptors

PPRE; peroxisome proliferator responsive element

PUFA; poly unsaturated fatty acids

q-RT-PCR; quantitative real-time reverse-transcription poly-chain reaction

RBC; red blood cells

RIA; radioimmunoassay

RNA; ribonucleic acid

SCD; stearoyl-CoA desaturase

SD; standard deviation

SFA; saturated fatty acids

sPLA; secretory phospholipase A

t; *trans*

*t*10,*c*12 CLA; *trans*10,*cis*12 18:2

*t*11; *t*11 18:1; vaccenic acid

*t*12; *t*12 18:1

*t*9; *t*9 18:1; elaidic acid

TAG; triacylglycerols

TE, tocopherol equivalents

*t*FA; *trans* fatty acids

TNFα; tumor necrosis factor α

TXB; thromboxan

UK; United Kingdom

US; United States

VCAM; vascular cell adhesion molecule

VLDL; very low density lipoprotein

w; women

wk; week

γ-GT; γ-glutamyltransferase

LIST OF MANUSCRIPTS

CHAPTER 2

Trans-11-18:1 is effectively Δ9-desaturated compared with _trans_-12-18:1 in humans.

Katrin Kuhnt, Jana Kraft, Peter Möckel, Gerhard Jahreis

British Journal of Nutrition 95, 752–61 (2006).

Content

The Δ9-desaturation of *t*11 and *t*12 in humans is presently unknown. In the present intervention study, related to the dietary intake of two *trans* isomers (*t*11 and *t*12 18:1) over a short-term (7 d) and a long-term (42 d) intervention period the concentrations of *t*11 and *t*12 were significantly increased in human lipids. The *t*11 was significantly Δ9-desaturated to *c*9,*t*11 CLA. Contrary to the *t*11, no Δ9-desaturation of *t*12 to *c*9,*t*12 18:2 could be observed. The mean conversion rate of *t*11 was approximately 24% in serum and 19% in red blood cell membranes.

WORK	CONTRIBUTION
Study accomplishment	100%
Analyses (lipids, diets)	90%
Statistics	100%
Preparation of the manuscript	100%

CHAPTER 3

Dietary supplementation with 11*trans*- and 12*trans*-18:1 and oxidative stress in humans.

Katrin Kuhnt, Andreas Wagner, Jana Kraft, Samar Basu, Gerhard Jahreis

American Journal of Clinical Nutrition 84, 981–8 (2006).

Content

Increased consumption of *t*FA has been associated with increased oxidative stress in humans. The oxidative stress marker 8-iso-$PGF_{2\alpha}$ (free radical induced) was significantly increased in the urine in relation to the supplementation of *t*11 and *t*12 18:1. The urine concentration of 15-ketodihydro-$PGF_{2\alpha}$ (COX-mediated) and 8-oxodG (oxidative DNA-damage) were unaffected. The anti-oxidative status in plasma (α-, β-, γ-, δ-tocopherol, retinol) was unchanged in subjects receiving *t*11 and *t*12 18:1.

WORK	CONTRIBUTION
Study accomplishment	100%
Analyses (biomarkers, anti-oxidative status, diets)	40%
Statistics	100%
Preparation of the manuscript	100%

CHAPTER 4

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, Jana Kraft, Heinz Vogelsang, Klaus Eder, Jürgen Kratzsch, Gerhard Jahreis

British Journal of Nutrition (2007), *accepted 3 January 2007, in press.*

Content

High *t*FA intake has been related to endothelial dysfunction and inflammation. With the supplementation of *t*11 and *t*12 18:1 in humans both *trans* isomers and the synthesized *c*9,*t*11 CLA increased significantly in lipids of peripheral blood mononuclear cells. The estimated *t*11 conversion rate was 18%. Despite the high intake of *t*FA (6.0 g/d) the determined inflammatory and immunological biomarkers such as interleukines, TNFα, CRP, adiponectin, ICAM-1, and prostacyclin as well as immune cell function (phagocytic process) remained unchanged.

WORK	CONTRIBUTION
Study accomplishment	100%
Analyses (immune system, biomarker, fatty acids)	40%
Statistics	100%
Preparation of the manuscript	100%

CHAPTER 5

Gender-specific gene expression in human monocytes in relation to dietary intervention with two *trans* fatty acids (*t*11 and *t*12 18:1).

Katrin Kuhnt, Silke Flotho, Sailas Benjamin, Torsten Börchers, Gerhard Jahreis, Friedrich Spener

American Journal of Clinical Nutrition, in preparation

Content

Fatty acids such as linoleic acid and CLA are potent ligands of gene regulators such as *peroxisome proliferator-activated receptors* (*PPAR*). Gene expression in monocytes was differentially regulated among gender in relation to the *t*11 and *t*12. In female test subjects the *cyclooxygenase 2* (*COX2*) and *fatty acid translocase* (*FAT*) whereas in male test subjects *PPARγ* and *glucose transporter* (*GLUT*) *4* were significantly increased. The observed effects can be attributed to *t*11 and/or *t*12 as well as to the endogenously synthesized *c*9,*t*11 CLA.

WORK	CONTRIBUTION
Study accomplishment	100%
Analyses (diets, monocytes isolation 90%; gene expression, 0%)	30%
Statistics (clinical data, diets 100%; gene expression, 0%)	50%
Preparation of the manuscript	60%

CHAPTER 1

INTRODUCTION AND OBJECTIVES

CHAPTER 1

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1 INTRODUCTION

The knowledge of the impact of the relation between dietary fatty acids and the risk of cardiovascular diseases, diabetes mellitus type-2, and cancer, the most common causes of death in developed countries increased in the last years.

Trans fatty acids (*t*FA) and conjugated linoleic acids (CLA) in the human diet have been of particular interest. In recent years, there is increasing evidence that *t*FA have detrimental effects on human health but with differences among positional isomers of *trans*-double bonds. In contrast, CLA show various metabolic properties, anti-cancerogenic, anti-inflammatory and anti-atherogenic, with potential impact in humans.

1.1 *TRANS* FATTY ACIDS

Structure of trans fatty acids

*t*FA are unsaturated fatty acids that have at least one double bond in *trans* configuration. In the *cis* configuration, the hydrogen atoms attached to the carbons are at the same side while in the *trans*-double bond, the hydrogen atoms are attached to the carbons in opposite directions. Fatty acids of the same chain length with double bonds in identical locations but with different configuration (*trans*/*cis*) are called geometric isomers like oleic acid and elaidic acid (*trans*9 18:1, *t*9); positional isomers are *t*9 and vaccenic acid (*trans*11 18:1, *t*11; FIGURE 1). The conformation of unsaturated fatty acids depends of the configuration of its double bonds. The *trans* orientation is of a straight shape, while the *cis* orientation gives a 30° rigid bend in the carbon chain.

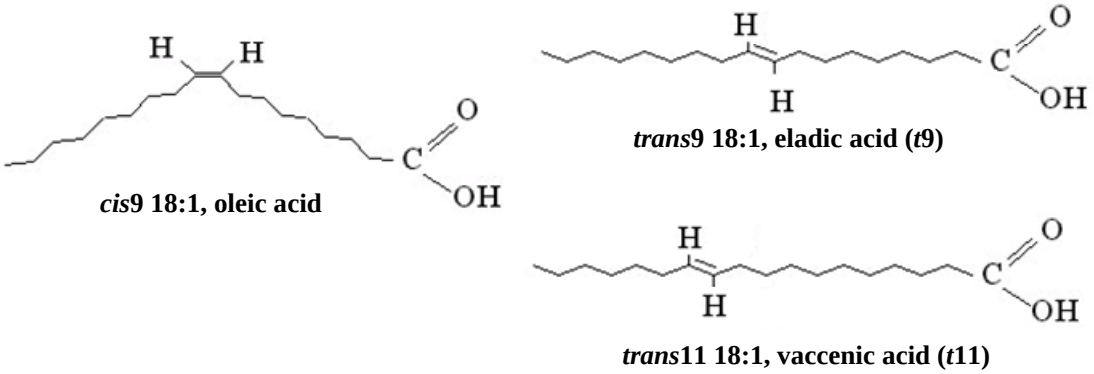


FIGURE 1 Structure of fatty acids with double bonds with *cis* and *trans* configuration.

Formation and occurrence of trans fatty acids

Most dietary unsaturated fatty acids are in *cis* orientation. The *t*FA in the diet originate from several sources.

Trans fatty acids are formed during

1. the hydrogenation of several unsaturated fatty acids by bacterial enzymes in the rumen of ruminant animals,
2. industrial processes such as partial hydrogenation and deodorization of vegetable oils high in PUFA, and
3. heating and frying of oils at extreme temperatures.

The goal of hydrogenation is to produce semi-solid and solid fats such as margarines and shortenings that are extensively used in many industrially prepared and processed foods (e.g., baked goods, snacks, etc.). The hydrogenation improves oxidation and thermal stability. Thus, partially hydrogenated oils are attractive for the food industry because of their long shelf life, stability during deep-frying, and their semi-solidity, which can enhance the palatability of baked goods and sweets.

The most common *t*FA in the diet are *trans* octadecenoic acids – *t*18:1 (50 - 90% of total [Σ] *t*FA; Steinhart *et al.* 2003) consisting of a large number of positional isomers (*t*4 to *t*16), but *trans* isomers of 14:1, 16:1, and PUFA (mainly *t*18:2 and *t*18:3) occur as well. Ruminant-derived products (milk and meat) contain generally low quantities of *t*FA (1 - 8% of Σ fatty acids; Aro *et al.* 1998, Precht *et al.* 2001). In contrast, industrially prepared foods contain high amounts of total fat mainly consisting of partially hydrogenated oils. These kinds of food products contain partly up to 60% *t*FA of Σ fatty acids, predominantly *trans* 18:1 isomers (Craig-Schmidt *et al.* 1998, unpublished data 2006, FIGURE 2).

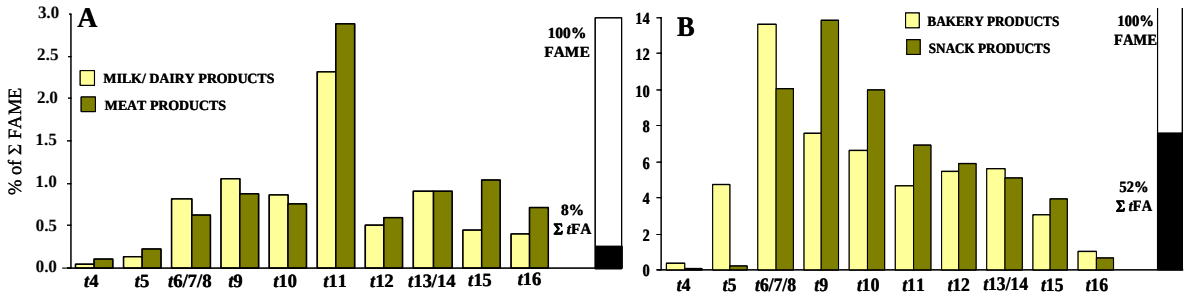


FIGURE 2 Distribution of *trans* isomers in lipids of foods from ruminant origin (A) and of industrially processed foods (B) [maximum value of each *trans* isomer; % of Σ FAME; unpublished data, 2006].

Both *t*FA sources contain the same *trans* isomers, but their isomeric profile clearly differes. In ruminant fats the *t*11 (FIGURE 2, A) is the predominant isomer (60 - 80% of Σ *t*FA) and the *t*9 is mostly less than 10% (Precht *et al.* 2001). In contrast, industrially hydrogenated fats and oils mainly contain the *t*6/7/8, *t*9, *t*10, *t*11, and *t*12 18:1 (*t*12) isomers (Aro *et al.* 1998; Kraft, 2006*a*; FIGURE 2, B).

Intake of trans fatty acids

Although numbers for the intake of *t*FA exist, they are only estimates, depending on analysis of food consumption, representativeness of the researched population, and especially of used laboratory analysis (Wolff *et al.* 2002). Intake estimates for dietary *t*FA differ from country to country. Current intake in the United States (US) and Canada is still higher compared to most European countries. In the US the average *t*FA consumption is about 2.6% of energy intake (en%; average 5.8 g/d; men 6.9 g/d, women 4.8 g/d; FDA 2003, TABLE 1). In Canadian women the average *t*FA intake was exceedingly high at 10.6 g/d (Chen *et al.* 1995). Interestingly, in the US approximately 80% of Σ *t*FA is currently derived from partially hydrogenated oils (Food and Drug Administration; FDA, 2003). In contrast, in the EU 60% derived from ruminant fats and 40% from partially hydrogenated fats. The daily intakes of *t*FA for 14 European different countries were estimated to range from 0.5 - 2.1 en% among men and 0.8 - 1.9 en% among women, respectively (0.9 en%; women 2.0 g/d, 1.0 en%; TRANSFAIR study 1995-1996; Hulshof *et al.* 1999, van de Vijver *et al.* 2000; TABLE 1). Intakes of *t*FA were lowest in the Mediterranean countries with 0.6 en% (Spain, Portugal, Greece) and highest in Iceland with 1.6 en%. According to analyses of German foods the estimated daily per capita intake was 1.9 g for women and 2.3 g for men (Fritsche & Steinhart, 1997). The daily intake of *t*11 in European countries ranges from 0.7 to 1.0 g/d (Voorrips *et al.* 2002). However, very few data from the intake of individual *trans* isomers, especially of the 18:1 are available.

In addition, the *t*FA content in human breast milk is an adequate biomarker for *t*FA intake. Samples from the US and Canada confirmed the highest *t*FA intake (7% by wt of Σ fatty acids; Chen *et al.* 1995, Mosley *et al.* 2005) and from China and Nigeria the lowest (0.6% and 1.2%; Chen *et al.* 1997, Koletzko *et al.* 1991). The breast milk of German women contained moderate amounts of *t*FA (4.2% by wt of Σ fatty acids; Koletzko *et al.* 1988; Precht & Molkentin, 1999).

Trans fatty acids - metabolism and relation to cardiovascular disease

*t*FA can be metabolized such as *cis* fatty acids by oxidation, elongation, and desaturation processes. They can be stored in adipocytes or can be incorporated into membrane lipids (Lemaitre *et al.* 2006).

Fatty acids can generally change properties of biological membranes, which are lipid bilayers made up of two lipophobic surfaces and a lipophilic core, in case of *t*FA to more rigid with lower fluidity. In addition, hydrophobic parts of membrane proteins interact with lipids of the bilayer. Conformational changes of membrane proteins depend on the structural differences of *cis* and *trans* configuration (Katz, 2002; FIGURE 3 II). In recent studies in animals *t*FA impairs cell membrane fluidity and insulin sensitivity (Saravanan *et al.* 2005). Furthermore, *t*FA can modulate enzyme activities, like Δ6- and Δ5-desaturase. *t*FA interfere with the metabolism of 18:2 *n*-6 and 18:3 *n*-3 (Mahfouz *et al.* 1980; Koletzko, 1991). The most important derivative of 18:2 *n*-6 is 20:4 *n*-6 and the most important derivatives of 18:3 *n*-3 are 20:5 *n*-3 and 22:6 *n*-3. In addition, *trans* isomers seem to displace the respective *cis* isomers from metabolic pathways. Thus, the availability of precursors for eicosanoid synthesis (prostaglandins, thromboxanes, and leukotrienes) is reduced and could disturb their balance (Stachowska *et al.* 2004*a*).

The high consumption of *t*FA is associated with the increased risk of several diseases such as cancer and diabetes mellitus type-2, however, strongest with the increased risk of cardiovascular disease. Cardiovascular disease is presently the leading cause of death and illness in developed countries. Atherosclerosis, a progressive disease characterized by the accumulation of lipids and fibrous elements in the arteries, constitutes the most important contributor of cardivascular disease. Abundant data link hypercholesterol- and triacylglycerolaemia to atherogenesis. However, recently it was appreciated that inflammatory mechanisms couple dyslipidaemia to atheroma formation. The expression of pro-inflammatory cytokines characterizes early atherogenesis and sytemic inflammation. Macrophages are involved in foam cell formation due to autonomous scavenger receptors (Libby, 2002; FIGURE 3I).

The main observed effect of high *t*FA consumption in humans was on the serum lipids and lipoprotein profile (Ascherio *et al.* 2006). Compared with SFA or *cis* unsaturated fatty acids the intake of *t*FA decreases HDL cholesterol, raises LDL cholesterol, increases the ratio of cholesterol to HDL cholesterol, and/or increases the serum TAG concentration (Mensink & Katan, 1990; Zock & Katan, 1992; Almendingen *et al.* 1995, Judd *et al.* 1994, 1998; Aro *et al.* 1997, Sundram *et al.* 1997, Ascherio *et al.* 1999, Lichtenstein, 1999; Mensink *et al.* 2003).

*t*FA can also modulate the activity monocytes and macrophages, as manifested by increased production of inflammatory mediators (FIGURE 3). In controlled studies in humans their intake was associated with systemic inflammation and endothelial dysfunction. In a study with women the greater intake of *t*FA was related to the increase of TNFα, and among the women with higher BMI, IL 6 and CRP were increased (Mozaffarian *et al.* 2004*a*). In overweight women, increased *t*FA intake was related to increased serum TNFα, IL 6, and CRP additionally to the increased concentrations of E-selectin and adhesion molecules ICAM-1 and VCAM-1 (Lopez-Garcia *et al.* 2005). Actually, in patients with heart disease *t*FA membrane levels were associated with higher concentrations of IL 6 and TNFα (Mozaffarian, 2004*b*). At present, the underlying subcellular mechanisms for these observed effects are not well established.

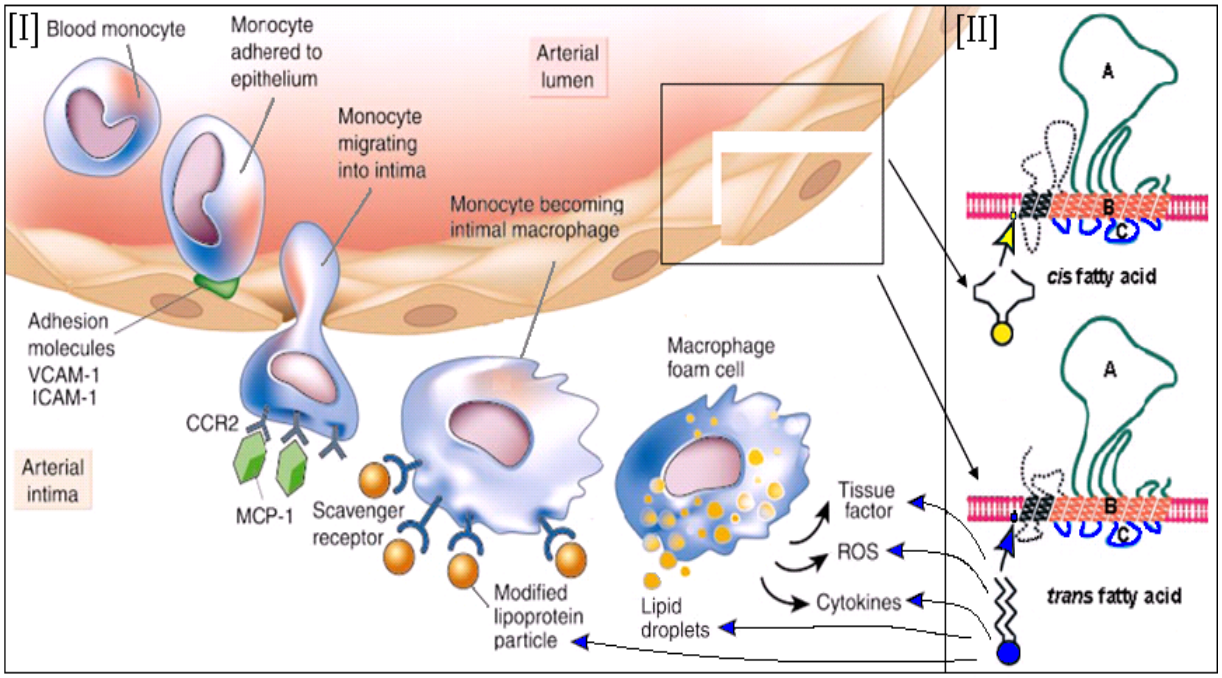


FIGURE 3 [I]: Mononuclear phagocytes in atherogenesis (modified from Libby, 2002). Panel [II]: Model of membrane proteins (A, cytosolic; B, transmembrane; C, extracellular) within a membrane lipid bilayer. Conformational changes of membrane proteins depending on *cis* and *trans* configuration of incorporated fatty acids (black dotted lines, modified from Katz, 2002). Incorporated and free *t*FA can influence oxidative stress, cytokine production, blood lipids and lipoproteins, and macrophage metabolism as well.

Mozaffarian and colleagues (2006) summarized from several prospective studies of *t*FA that a 2% increase in the energy intake from *t*FA was associated with a 23% increase in the incidence of cardiovascular disease (pooled relative risk 1.23: Health Professionals Follow-up Study, Ascherio *et al.* 1996; Alpha-Tocopherol Beta-Carotene Caner Prevention Study, Pietinen *et al.* 1997; Zutphen Eldery Study, Oomen *et al.* 2001; Follow-up Nurses Health study, Oh *et al.* 2005). This is in agreement with the estimation of Hu *et al.* (2001) from the

US Nurses Health study. The meta-analysis of 60 controlled trials of Mensink *et al.* (2003) concluded risk is reduced most effectively when *t*FA and SFA are substituted with *cis* unsaturated fatty acids. However, no association of European intake levels and unfavorable serum profile could be conclude from data from the TRANSFAIR study (van de Vijver *et al.* 2000).

The relation of *t*FA concentrations in several tissues, adequate biomarkers of *t*FA intake, and the risk of cardiovascular disease have been examined in retrospective case-control studies (Chapter 6, TABLE 7). Hodgson *et al.* (1996) suggested several separate *trans* isomers of 18:1 are differently associated to the risk of cardiovascular disease. There are moreover justified indications that *trans* classes such as 18:1 and 18:2 have different impacts on the risk of cardiovascular disease. It becomes apparent that a high *trans* 18:2 content in membranes was associated with increased infarction risk. Contrary, the *trans* 18:1 content was negatively associated with risk (Baylin *et al.* 2003; Lemaitre *et al.* 2002, 2006). Unfortunately, further studies, which compare effects of single *trans* isomers and *trans* classes, are missing in humans. On that account differences in the consumption of industrial-derived and ruminant-derived *trans* fat, which display different *trans*-isomer profiles, indicate different risks of cardiovascular disease.

Reducing the intake of trans fatty acids

As a result, numerous health professional groups have recommended that the public reduce their consumption of *t*FA as well as SFA. Canada, as a country with one of the highest *trans* fat intake became the first country to pass legislation requiring mandatory declaration of the *t*FA content of foods (effective December 2005, Health Canada). In January 2003, the FDA ruled in the US that the nutrition labels must indicate the content of *t*FA (FDA, 2003; effective January 1, 2006). In Europe, only the Danish government in 2004, mandated that all oils and fats used in locally made or imported foods must contain less than 2% industrially produced *t*FA (Stender & Dyerberg, 2004). Currently, researchers from the University of Oxford are claiming that food labels should list *trans* fats as well as cholesterol and saturated fats (Clarke & Lewington, 2006). However, the UK Food Standards Agency is currently revise of the European directive that governs the content and format of nutrition labels on foods marketed in UK and other European countries, so that *t*FA are labeled (Clarke & Lewington, 2006).

The food industry has recently lowered the content of *t*FA and many of the fast food restaurants switched from solid partially hydrogenated oil to liquid oils, which have lower

amounts of *t*FA (Fritsche & Steinhart, 1998; Stender, 2006). However, in the US and Canadian population the *t*FA intake has been relatively constant at 5.3 g/d to 8.4 g/d (Continuing Survey of Food Intakes by Individuals, Allison *et al.* 1999; TABLE 1). In contrast, in European countries a continuous decrease of *t*FA intake could be observed. In Germany, it decreased from 3.8 g/d in 1992 (Steinhart & Pfalzgraf, 1992) to 2.1 g/d in 1997 most likely due to the reduced *t*FA contents in German margarines (Fritsche & Steinhart, 1997). In the UK, *t*FA intake decreased from 7.0 g/d in 1984 to 1.9 g/d in 1997 (TABLE 1).

TABLE 1

Comparison of the *t*FA consumption between different countries and time periods.

YEAR	US	Canada	European countries			
			UK	The Netherlands Scandinavia	Germany	Mediterranean countries
		9.1[7]	**5.6**[10]		**5.6**[18]	**0.3**[21]
1985	**7.6**[1a]		**7.0**[11]	**10**[15]		
1990	**8.1**[1b] **12.8**[2] **5.3**[3]		**5.0**[10]	**2.5**[16]	**3.8**[19]	
	5.6[5]	**10.6**[8]	**4.8**[10]	**4.7**[17]		
1995	**5.8**[4] **5.3**[6]	**8.4**[9]	**2.8**[14] **1.9**[12] **3.1**[13]	**2.7**[14*] **4.8**[14**]	**2.4**[14] **2.1**[20]	**1.6**[14†]
2000				**1.7**[22]		

[1] a.) 1984, b.) 1989, Hunter & Applewhite, 1991; [2] Enig *et al.* 1990; [3] Continuing Survey of Food Intakes by Individuals (CSFII) 1989-91, [4] CSFII 1994-96, [5] National Health and Nutrition Examination Survey (NHANES III) 1988-94; FDA 2003; [6] 1995, Allison *et al.* 1999; [7] 1977, Brisson 1981; [8] 1992, Chen *et al.* 1995; [9] Ratnyake *et al.* 1998; [10] British Nutrition Foundation 1995; [11] Burt & Buss, 1984; [12] Ministry of Agriculture, 1997; [13] Wolff *et al.* 1995; [14] TRANSFAIR study 1995-96; Hulshof *et al.* 1999; * mean Denmark, Finland, Sweden; ** Norway; † mean Spain, Portugal, Greece, Italy; [15] The Netherlands 1985, van Dokkum *et al.* 1989; [16] The Netherlands 1986-1992, Voorrips *et al.* 2002; [17] The Netherlands, Wolff *et al.* 1995; [18] estimated by data from Heckers *et al.* 1978; [19] Steinhart & Pfalzgraf, 1992; [20] Fritsche & Steinhart, 1997; [21] Greece 1958-64, Kromhout *et al.* 1995; [22] Jakobsen *et al.* 2006*a*, from ruminant *t*FA in Danish population.

Ruminal and endogenous synthesis of trans fatty acids

Various *trans* isomers of 18:1 are formed by the incomplete biohydrogenation from several PUFA such as linoleic acid and linolenic acids in the rumen involving microbial enzymes (FIGURE 4). The major *trans* 18:1 isomer is the *t*11, especially ruminally formed from the *cis*9,*trans*11 18:2 (*c*9,*t*11 CLA). The opposite formation pathway of *c*9,*t*11 CLA from *t*11 as precursor by Δ9-desaturation was proposed (Pollard, 1980; Holman & Mahfouz, 1981).

The Δ9-desaturase (stearoyl-CoA desaturase, SCD; EC 1.14.99.5) catalyzes the insertion of a *cis*-double bound in the Δ9 position. The preferred substrates are palmitoyl-CoA (16:0) and stearoyl-CoA (18:0), which are converted to palmitoleoyl-CoA (16:1) and oleyl-CoA (18:1) (Mahfouz *et al.* 1980). The oxidative reaction is catalyzed by a set of microsomal electron-transport proteins composed sequentially of NADH cytochrome b_5 reductase, iron containing cytochrome b5, and the terminal Δ9-desaturase. Δ9-desaturase is the rate-limiting component in this reaction (Ntambi, 1999; FIGURE 4).

The Δ9-desaturase can insert a double bond in the Δ9 position in the vaccenoyl-CoA (*t*11 CoA) as well. Ruminant-derived *t*11 can be utilized in several tissues and can be convert to *c*9,*t*11 CLA (FIGURE 4). The endogenous synthesis of *c*9,*t*11 CLA from ruminal *t*11 by the Δ9-desaturation in tissues was discovered as the major source (70 - 95%) of CLA in milk fat (Griinari *et al.* 2000, Piperova *et al.* 2002, Mosley *et al.* 2006*a*).

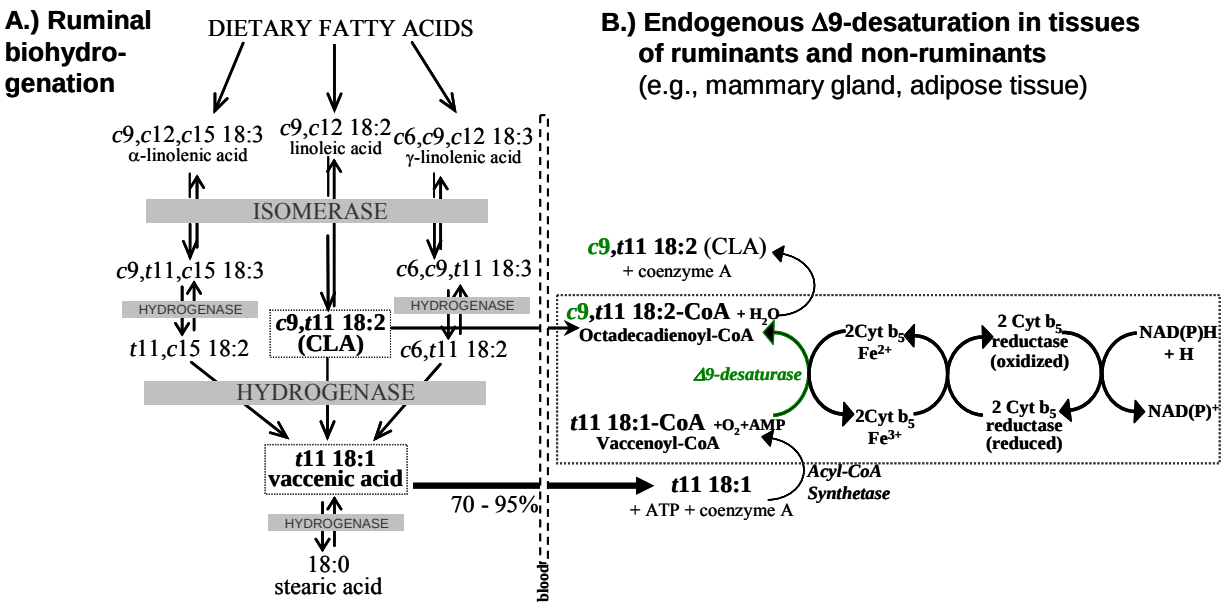


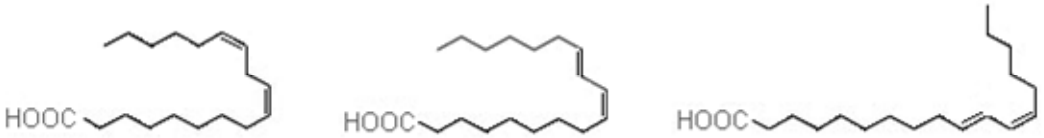
FIGURE 4 Biosynthesis of *t*11 18:1 (vaccenic acid) and *c*9,*t*11 18:2 (*c*9,*t*11 CLA) during the ruminal biohydrogenation and the endogenous Δ9-desaturation (modified from Griinari *et al.* 2000; Ntambi, 1999).

This endogenous CLA synthesis from dietary *t*11 was documented in non-ruminant animals such as mice, rats, and pigs (Santora *et al.* 2000, Banni *et al.* 2001; Loor *et al.* 2002, Gläser *et al.* 2002, Kraft *et al.* 2006*b*) but poorly evaluated in humans. Turpeinen and colleagues (2002) observed in humans an increase of *c*9,*t*11 CLA after a one week *t*11-rich diet. No study is available considering the Δ9-desaturation of other *trans* 18:1 isomers in humans.

1. 2 CONJUGATED LINOLEIC ACIDS

Structure of conjugated linoleic acids

Double bonds of unsaturated fatty acids can be positioned in either a non-conjugated or a conjugated arrangement. The most common form of double bonds is the non-conjugated that are separated by a methylene group (CH_2). Conjugated double bonds are coupled. The double bond pairs are adjacent to each other with no interceding CH_2 group (FIGURE 5).



***cis*9,*cis*12 18:2**
linoleic acid

***cis*9,*trans*11 18:2**
***c*9,*t*11 CLA**

***trans*10,*cis*12 18:2**
***t*10,*c*12 CLA**

FIGURE 5 Structure of fatty acids with non-conjugated and conjugated double bonds.

Formation of conjugated linoleic acids

Ruminant fat is the only significant source of CLA. During the hydrogenation of PUFA by various rumen bacteria numerous geometric (*t*/*t*, *c*/*t*, *t*/*c*, and *c*/*c*) and positional (2,4 to 14,16) isomers of conjugated linoleic acids (CLA) are created. A total of 54 CLA isomers are possible (Delmonte *et al*. 2004). The *c*9,*t*11 CLA was identified as an intermediate of linoleic acid biohydrogenation in the rumen by the bacterium *Butyrivibrio fibrisolvens* (Kepler *et al.* 1966, FIGURE 4). The first identified responsible enzyme was the linoleic acid isomerase (EC 5.3.1.5). The *c*9,*t*11 CLA is the most naturally abundant CLA isomer and accounts for 75 to 90% of Σ CLA present in ruminant fat (Parodi, 2003). The majority of *c*9,*t*11 CLA in milk fat is synthesized endogenously from *t*11 in the mammary gland (FIGURE 4). As a result the milk fat content of *t*11 and *c*9,*t*11 CLA generally changes in concert and approximate a 3:1 ratio (Bauman *et al.* 2003).

Further relevant CLA in ruminant fat are the *t*7,*c*9 and *t*11,*c*13 isomers and represent 3 to 16% and 2 to 8% of Σ CLA, respectively (Fritsche *et al.* 2000, Yurawecz *et al.* 2002, Kraft *et al.* 2003). The *t*7,*c*9 CLA in ruminant fat is almost exclusively originated from endogenous Δ9-desaturation of ruminant-derived fatty acids (Corl *et al.* 2002, Piperova *et al.* 2002). Milk fat is the major dietary source of CLA containing 3 to 37 mg CLA/g fat (Kraft *et al.* 2003). The CLA content and isomeric pattern is dependent on season, feeding regiment, species, and stalling time (Jahreis *et al.* 1999).

Intake of conjugated linoleic acids

The estimation of CLA intake differs like the *t*FA intake from the habitual diet of individuals, different cultures, and used evaluation and analytical methods. In Germany CLA intake ranges from 0.3 to 0.5 g/d (Fritsche & Steinhart, 1998; Fremann *et al.* 2002) while in US and Canada the CLA intake is lower and varies from 0.1 to 0.2 g/d (Ritzenthaler *et al.* 2001, Ens *et al.* 2001). Voorrips *et al.* (2002) estimated a generally low CLA intake with 0.2 g/d in females of the Netherlands Cohort Study.

CLA contain *trans*-double bonds, except the *c*/*c* isomers, and are therefore *t*FA. However, considering the observed beneficial effects of CLA some countries have excluded CLA from their definition of *t*FA. This is relevant for the purpose of food labeling. The US and Canada exclude CLA from their definition of *t*FA whereas, however, the Australian and New Zealand Foods Standards Agencies definition will not exclude the CLA (Booker & Mann, 2005; Ratnayake & Zehaluk, 2005).

Effects of conjugated linoleic acids

CLA showed unique properties in animal models including effects on body composition and anti-cancerogenic, anti-atherogenic, and immunmodulatory effects (TABLE 2). Although the *trans*10,*cis*12 18:2 (*t*10,*c*12 CLA) is a minor CLA isomer in ruminant-derived fats (~1.1% of Σ CLA) the commercial CLA supplements are isomeric mixtures, containing *c*9,*t*11 and *t*10,*c*12; in equal amounts. There are indications that the isomers have different biological actions, although most of the human studies used mixtures of the synthetically prepared CLA. Relatively pure isomers are now available and allow determining isomer-specific effects (Lin *et al.* 2004, Song *et al.* 2006). The observed effects of CLA in humans are generally inconsistent and less significant than in animals.

- *Effects of conjugated linoleic acids on body composition*

Most animal studies have shown that CLA affect body composition. High isomer-specific effects were shown. The *c*9,*t*11 CLA is the active anabolic agent responsible for weight gain whilst the *t*10,*c*12 CLA is the effective catabolic agent leading to increased lipolysis and fat oxidation (Keim, 2003). The extent to which CLA are effective in changing body composition varies according to the species whereby mice are the most responsive (TABLE 2).

However, studies in humans have shown no effects, possibly depending on the received CLA dosage and study duration (Wang & Jones, 2004). Only in obese and overweight humans decreases in body fat have been observed (Blankson *et al.* 2000, Risérus *et al.* 2001).

- *Anti-cancerogenic effects of conjugated linoleic acids*

Anti-cancerogenic effects of CLA have been demonstrated in different organs (e.g., skin, mammary gland, prostate, and fore stomach) in several experimental cell culture models and also animal models, which are inhibiting tumor development at initiation, promotion, and progression (Lee *et al.* 2005). Mechanisms of action could include the reduction of cell proliferation, induction of apoptosis, and increased lipid peroxidation (Schonberg & Krokan, 1995; O´Shea *et al.* 1999; Ip *et al.* 2000, 2001; Belury, 2002; Masso-Welch *et al.* 2002, Miller *et al.* 2002, Song *et al.* 2006, Cho *et al.* 2006). CLA are potent activators of peroxisome proliferator-activated receptors (PPAR) α and γ (Moya-Camarena *et al.* 1999, Belury *et al.* 2002). PPAR regulate lipid and lipoprotein metabolism, cell proliferation, differentiation, and apoptosis (Kersten *et al.* 2000). CLA dependent suppression of cytokine production is related to PPAR activation (Yu *et al.* 2002, TABLE 2). The anti-tumor action of CLA appears to be more efficient in mammary gland than in other organs.
However, one of three epidemiological studies in humans showed a significant negative association between *c*9,*t*11 CLA and the risk of breast cancer (Aro *et al.* 2000).

- *Anti-atherogenic and anti-inflammatory effects of conjugated linoleic acids*

In general, CLA modify membrane composition, enzyme activity, and eicosanoid synthesis (Eder *et al.* 2003). CLA were shown to reduce eicosanoid precursors such as arachidonic acids in membranes and inhibit prostaglandin synthesis in vascular smooth muscle cells (Ringseis *et al.* 2006). Further, inhibitory effect of CLA on pro-inflammatory cytokines (e.g., IL 8) was observed in cell models, most likely mediated through its interaction with PPARγ (Ringseis *et al.* 2006, Jaudszus *et al.* 2005; TABLE 2).
CLA have been shown to have no major effects on plasma lipids in humans (Terpstra *et al.* 2004). In various studies significant effects of CLA, e.g. TAG decrease and HDL increase disappeared when compared to control group (Smedman & Vessby, 2001; Mougious *et al.* 2001). Tricon *et al.* (2004) showed obviously opposing effects of *c*9,*t*11 and *t*10,*c*12 CLA on serum lipids.

➢ *Immunmodulatory effects of conjugated linoleic acids*

There is some evidence that CLA can enhance several specific immune functions. For example, CLA could improve the formation of protective immunoglobulins (Ig) while IgE was reduced (Bassaganya-Riera *et al.* 2003, Sugano *et al.* 1998). In addition, CLA could be beneficial in certain types of allergic or inflammatory responses (O´Shea *et al.* 2004).
Two human studies with CLA regarding the immune system were not able to show any effects of CLA (Albers *et al.* 2003, Kelley *et al.* 2001).

TABLE 2

Reported beneficial effects of conjugated linoleic acids observed in animal and cell models.

	Main effect	**Possible underlying mechanisms**
Modulation of body composition	Increase of lean body mass	Lipolysis ↑, LPL ↑, CPT ↑
	Decrease of body fat mass	Fat mobilization and oxidation ↑
	Reduced fat deposition	Adipocyte proliferation ↓, Leptin ↓
Anti-cancerogenic	Inhibition of cell proliferation	Alteration of cell cycle proteins (cyclin D1, A)
	Inhibition of tumor growth/ metastasis, angiogenesis	Expression *bcl-2* ↓, *bax* ↑, *bcl-xs* ↑, *p53* ↑
		Lipid peroxidation in carcinoma cells ↑
	Induction of apoptosis	Suppression of cytokines by PPARα, γ activation and NFκB inhibition
	Cytotoxicity	
Anti-inflammatory/ Anti-atherogenic	Reduced plaque formation	Membrane composition (AA ↓)
	Inhibition of cytokine formation, angiogenesis, and formation of adhesion molecules	Eicosanoid synthesis (PGE_2 ↓, PGI_2 ↓)
		Enzyme activity ↓ (COX2, LOX5,-12,-15, PLA_2)
		PPARα, γ ↑; cytokines ↓ (IL 1, IL 6, TNFα)
Modulation of immune function	Stimulation adaptive immune response	Cytokine production ↓ (IL 8)
		IgA, IgG, IgM ↑, IgE ↓
	Increase of immune cell proliferation	$CD8^+$ T-cells ↑, NK ↑

NF, nuclear factor; LPL, lipoprotein lipase; CPT, palmitoyl carnitine acyl transferase; NK, natural killer cells.
Reviewed in: Parodi, 2003; Terpstra, 2004; Wahle *et al.* 2004, Lee *et al.* 2005.

➢ *Adverse effects of conjugated linoleic acids*

Reports of detrimental effects of CLA intake appear to be largely in mice, mainly due to the *t*10,*c*12 isomer regarding insulin resistance, fatty liver, and inflammation (Poirier *et al.* 2006, de Roos *et al.* 2005; Wang & Jones, 2004). Detrimental effects of the *t*10,*c*12 isomer were indicated in humans as well (Smedman *et al.* 2005, Risérus *et al.* 2002 *a,b*).

1.3 OBJECTIVES

The previous data support a relationship between high *t*FA intake and risk of cardiovascular disease but most likely with differences among positional isomers of *trans*-double bonds and the type of double bonds. Very few data are available referring the metabolism and physiologic effects of single *trans* 18:1 isomers.

The present studies were conducted to contribute data regarding the Δ9-desaturation of two simultaneously supplemented *trans* isomers *t*11 and *t*12 18:1 to their respective products *c*9,*t*11 CLA and *c*9,*t*12 18:2. The Δ9-desaturation was determined in healthy male and female subjects on different dosages and supplementation periods.

Furthermore, the incorporation of both supplemented substrates and their products into human tissue lipids (serum, membranes of red blood cells and peripheral blood mononuclear cells) was determined.

For clarifying the effects of the *trans*-isomer intervention on healthy humans the serum lipids and lipoproteins were determined and various biomarkers of inflammation, oxidative stress, and immune system were examined. In addition, the gene expression due to the dietary intervention was investigated in isolated monocytes of study subjects (FIGURE 6).

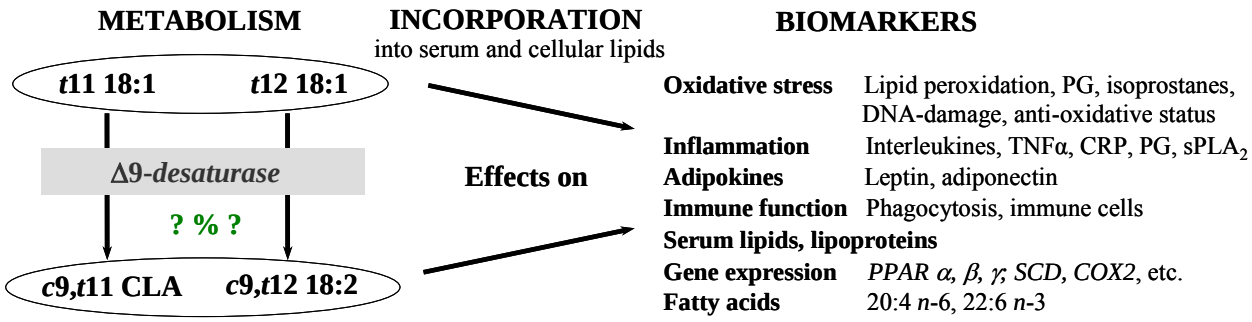


FIGURE 6 Objectives of the present intervention studies.

CHAPTER 2

Trans-11-18:1 is effectively Δ9-desaturated compared with *trans*-12-18:1 in humans.

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Trans-11-18 : 1 is effectively Δ9-desaturated compared with *trans*-12-18 : 1 in humans

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The aim of this human intervention study was to evaluate the Δ9-desaturation of *trans*-11-18 : 1 (*trans*-vaccenic acid; *t*VA) to *cis*-9,*trans*-11-18 : 2 (*c*9,*t*11 conjugated linoleic acid; CLA) and of *trans*-12-18 : 1 (*t*12) to *cis*-9,*trans*-12-18 : 2 after a short-term (7 d) and a long-term (42 d) supplementation period. The conversion rates of both *trans*-18 : 1 isomers were estimated by lipid analysis of serum and red blood cell membranes (RBCM). Subjects started with a 2-week adaptation period without supplements. During the 42 d intervention period, the diet of the test group was supplemented with 3 g/d of *t*VA and 3 g/d of *t*12. The diet of the control group was supplemented with a control oil. Serum *t*VA and *t*12 levels in the test group increased by fivefold and ninefold after 7 d, respectively, and by eight- and 12-fold after 42 d, respectively, when compared with the adaptation period ($P \leq 0{\cdot}002$). The serum *c*9,*t*11 CLA levels increased by 1·7- and 2·0-fold after 7 d and 42 d, respectively ($P \leq 0{\cdot}001$). After 42 d, the test group's RBCM *c*9,*t*11 CLA content was elevated by 20 % ($P = 0{\cdot}021$), whereas in the control group it was decreased by 50 % ($P = 0{\cdot}002$). The conversion rate of *t*VA was estimated at 24 % by serum and 19 % by RBCM. No increase in *c*9,*t*12-18 : 2 was observed in the serum and RBCM, and thus no conversion of *t*12 could be determined. In conclusion, the endogenous conversion of dietary *t*VA to *c*9,*t*11 CLA contributes approximately one quarter to the human CLA pool and should be considered when determining the CLA supply.

Conjugated linoleic acids: *trans*-Vaccenic acid: *trans*-12-18 : 1: Δ9-Desaturation: Man

Trans-vaccenic acid (*trans*-11-18 : 1; *t*VA) is the predominant *trans* monoen in ruminant fats (30–80 % of total *trans*-18 : 1 isomers; Aro *et al.* 1998; Precht *et al.* 2001; unpublished data), depending on the cattle feeding regime (Jahreis *et al.* 1997; Bauman & Griinari, 2003; Kraft *et al.* 2003). In partially hydrogenated vegetable oils, *t*VA ranges between 13 % and 22 % of total *trans*-18 : 1 fatty acid (Molkentin & Precht, 1995; Aro *et al.* 1998; Wolff *et al.* 2000). The content of *trans*-12-18 : 1 (*t*12) is similar in both and ranges from 4 % to 13 % of total *trans*-18 : 1 (Kraft *et al.* 2003; European Food Safety Authority, 2004).

*t*VA is formed during the biohydrogenation of several PUFA (e.g. *c*9,*c*12-18 : 2) by rumen bacteria (Noble *et al.* 1974). In this process, numerous geometric and positional isomers of conjugated linoleic acid (CLA) are created as further intermediates, but *cis*-9,*trans*-11-18 : 2 (*c*9,*t*11 CLA) is predominantly formed (Kepler *et al.* 1966; Griinari & Bauman, 1999). The endogenous desaturation of both *t*VA to *c*9,*t*11 CLA and *t*12 to *c*9,*t*12-18 : 2 is catalysed by stearoyl-CoA desaturase (E 1.14.99.5), also commonly known as Δ9-desaturase (Pollard *et al.* 1980; Holman & Mahfouz, 1981; Griinari *et al.* 2000). In cows, the endogenous synthesis of *c*9,*t*11 CLA from *t*VA occurs mainly in the mammary gland and accounts for the main source of *c*9,*t*11 CLA in the milk and tissues (Griinari *et al.* 2000; Corl *et al.* 2001; Piperova *et al.* 2002). The conversion of *t*12 to *c*9,*t*12-18 : 2 in humans is still, however, unknown.

Several studies have provided evidence for the endogenous synthesis of CLA via Δ9-desaturase in non-ruminant animals (Ip *et al.* 1999; Gläser *et al.* 2000; Santora *et al.* 2000; Banni *et al.* 2001; Loor *et al.* 2002; Kraft, 2004), as well as in man (Salminen *et al.* 1998; Adlof *et al.* 2000; Turpeinen *et al.* 2002). The enrichment of CLA in body tissues via the endogenous conversion of *t*VA was associated with anticarcinogenic effects in animals (Ip *et al.* 1999; Banni *et al.* 2001; Corl *et al.* 2003; Lock *et al.* 2004). These researchers and others have postulated potential effects for CLA, and these concepts have been reviewed in Belury (2002), Parodi (2004) and Lee *et al.* (2005).

Wolff (1995) reported dietary intake levels of 1·3–1·8 g/d of total *trans*-18 : 1 from ruminant fats for people from countries of the European Economic Community (except Spain and Portugal). Thus, the daily intake of *t*VA in the most European countries probably exceeds 0·7–1·0 g/d, whereas the CLA dietary intake is lower, ranging between 300 and 500 mg/d (Fritsche & Steinhart, 1998; Aro *et al.* 2000; Fremann *et al.* 2002; Jahreis & Kraft, 2002; Voorrips *et al.* 2002). At present, insufficient data are available concerning the isomeric distribution of *trans*-18 : 1 in different food sources, and the human dietary intake of these individual isomers is generally unknown.

The aim of the present human intervention study was to evaluate the endogenous Δ9-desaturation of both *t*VA (3·0 g/d) to *c*9,*t*11 CLA, and *t*12 (3·0 g/d) to *c*9,*t*12-18 : 2,

Abbreviations: *c*, *Cis* fatty acid; CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; RBC, red blood cells; RBCM, red blood cell membranes; *t*, *trans* fatty acid; *t*12, *trans*-12-18:1; TAG, triacylglycerol; *t*VA, *trans*-vaccenic acid.

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after a short-term (7 d) and a long-term (42 d) supplementation period. The conversion rates of the two *trans*-18 : 1 isomers were estimated by lipid analysis of serum and red blood cell membranes (RBCM).

Subjects and methods

Subjects

Volunteers were recruited by advertisement. The volunteers were selected after confirming that they were healthy, had a BMI of over 18 kg/m^2 and less than 30 kg/m^2, had no diagnosed diseases, were not taking any medications (except contraceptives), were not vegetarians or vegans, were not abuser of alcohol and were taking no dietary supplements. Women and men fitting these criteria were informed of the purpose, course and possible risks of the study. All volunteers completed a questionnaire on health, lifestyle and dietary factors (e.g. consumption of dairy products) before entering the study. All subjects gave their written informed consent before participating. The study protocol was approved by the Ethical Committee of the Friedrich Schiller University of Jena. Body fat measurements were performed using a 50 kHz-frequency impedance analyser (Data Input GmbH, Darmstadt, Germany) with phase-sensitive technology. Bioelectrical impedance assessment and body weight were recorded at the beginning of the study and at the end of each study period.

The age of the volunteers ranged from 20 to 28 years (mean 24 (SD 3 years), and the BMI were between 19 and 26 kg/m^2 (mean 21 (SD 2) kg/m^2). Two subjects were smokers (< ten cigarettes/d), and all the women were taking oral contraceptives. The subjects were normocholesterolaemic (mean 4·4 (SD 0·7) mmol/l) and had an LDL-cholesterol:HDL-cholesterol ratio of less than 3 and a triacylglycerol (TAG) concentration of 1·0 (SD 0·4) mmol/l (Table 1).

Study design and diets

The study consisted of a 2-week adaptation period and a 6-week intervention period. Throughout the complete study (8 weeks), the volunteers had to consume a ruminant-fat-free baseline diet. During this time, the volunteers consumed their habitual diet but without ruminant-related products (meat, meat products, milk, dairy products), thus minimizing their intake of CLA and *trans*-fatty acids. They were instructed to substitute the staples of soya milk for milk, margarine for butter, vegetable coffee whitener for cream, vegetable spreads for cheese, etc. in their habitual diet. In addition, the subjects received recipes to prepare ruminant-fat-free meals, cakes, etc. to comply with the baseline diet.

At the end of each study period, the volunteers consumed a standardized ruminant-fat-free diet over the last 7 d (Fig. 1). During the standardized diet, the volunteers received fresh food every day and were instructed to consume only the provided food. All components of the diet were identical for each participant and were consumed to their individual requirements.

After the adaptation period, the subjects were randomly allotted to two groups (twelve per group). As a criterion for selecting subjects, the number of men and women was balanced in each study group: six men and six women. Before the intervention study was started, the characteristics of the two treatment groups, for example anthropometric data, LDL-cholesterol:HDL-cholesterol ratio, TAG concentration and total cholesterol, were compared to confirm optimal study group selection (Table 1). The diet of the test group was supplemented with 3·0 g/d of *t*VA and 3·0 g/d of *t*12. The control group diet was supplemented with a control oil to make the diets of the two treatment groups isocaloric (Fig. 1).

A commercially prepared mixture of fatty acids (Natural ASA, Hovdebygda, Norway) was used for this study because of its availability and reasonable costs. This *trans*-isomer mixture comprised mainly *t*VA and *t*12 (1-1), and these two components constituted over 60 % of the total fatty acids in the preparation. In addition, the *trans*-isomer mixture contained approximately 20 % total fatty acids of *c*11-18 : 1 and *c*12-18 : 1 in equal shares as technical byproducts. This *trans*-isomer mixture was applied as a TAG. The control oil was a mixture of palm kernel oil and rapeseed oil in the ratio 1:1, which possesses a fatty acid distribution similar to that found in the common Western diet without *trans*-fatty acids and CLA.

Both experimental fats (*trans*-isomer mixture and control oil) were added to a commercially available chocolate spread (with *c*9 -18 : 1 as its predominate fatty acid) to make the supplements palatable to the volunteers. During the adaptation period, the volunteers consumed 20 g/d of the pure chocolate spread. In the intervention period, both groups consumed daily 20 g of the experimental fat/chocolate spread mixture (with control oil or *trans*-isomer mixture, depending on the group).

Before starting the study, the energy requirements of the each individual subject were determined by recording the total individual dietary intake for a 7 d period. Standardized diet food supplies were provided to meet the individual subject's

Table 1. Characteristics of the treatment groups before the intervention period

(Mean values and standard deviations)

	Control group (*n* 12)		Test group (*n* 12)	
Parameter	Mean	SD	Mean	SD
Age (years)	24	3	25	2
Body weight (kg)	63	12	66	13
Body height (cm)	174	12	177	12
BMI (kg/m^2)	21	2	21	2
Body fat mass (%)	18	6	20	6
Total cholesterol (mmol/l)	4·1	0·6	4·5	0·8
LDL- cholesterol : HDL- cholesterol	1·8	0·6	2·1	0·7
Triacylglycerol (mmol/l)	1·0	0·4	1·0	0·4

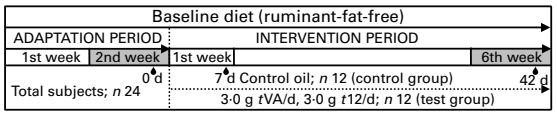

Fig. 1. Design of the intervention study. During an 8-week ruminant-fat-free baseline diet, twelve subjects (test group) received a *trans*-isomer mixture over 42 d, and twelve subjects received a control oil free of *trans*-fatty acids and CLA (control-group); ● blood sampling, ■ standardized diet over 7 d. *t*VA, *trans*-vaccenic acid; *t*12, *trans*-12–12 : 1.

requirements. The data provided by the 7 d food intake record were analysed using the PRODI 4.4 expert software package (Nutri-Science GmbH, Freiburg, Germany). During the time the standardized was consumed, the residues and non-comestibles (e. g. banana peel) of the provided food were returned and weighed each day, thus allowing for more accurate determinations of food consumption. Duplicate portions of the dietary supplies were collected, homogenized and sampled to allow for nutritional analysis of the study diet. The homogenized samples were freeze-dried, and DM, total fat and N content were determined according to the methods of the Association of Official Analytical Chemists (1995). The total dietary fibre content was analysed by an enzymatic test kit (BIOQUANT; Merck, Darmstadt, Germany). The total digestible carbohydrates were calculated as the difference between the DM and the sum of protein, fat and dietary fibre.

Blood collection

Blood samples were collected after 7 d of the standardized diet had been consumed for both the adaptation (0 d) and intervention (42 d) periods. In addition, blood was collected on day 7 of the intervention period (Fig. 1).

After an overnight fast, blood was collected between 07.30 and 08.30 hours by venepuncture into Vacutainers for serum preparation. Red blood cells (RBC) were isolated from blood collected into Vacutainer (BD Vacutainer Systems, Heidelberg, Germany) tubes with EDTA as an anticoagulant. After the plasma and platelets had been removed (15 min, 1000 *g*), the RBC were dispersed in PBS (0·9 %) and washed three times by centrifugation (20 min, 1000 *g*). After freezing at −80°C, membrane preparations were washed two or three times in PBS (0·9 %) until the supernatant was clear in order to remove haemoglobin and other cytoplasmic components.

Cholesterol determination

Serum total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG concentration were ascertained by enzymatic methods using the autoanalyser Synchron LX system (Beckman Coulter, Fullerton, USA).

Lipid analysis

The lipid contents of serum, RBCM and food samples were extracted with chloroform–methanol–water (2:1:1, v/v/v) according to Folch *et al.* (1957). The lipid extracts of RBCM and food were concentrated and treated with $NaOCH_3$ (0·5 M $NaOCH_3$ in methanol, 15 min, 60°C) to produce fatty acid methyl esters (FAME) extracts. FAME of serum lipids were prepared by using a combination of $NaOCH_3$ and 1,1,3,3-tetramethylguanidine (Sigma-Aldrich, St Louis, USA; 1,1,3,3-tetramethylguanidine in dry methanol, 1:4, v/v, 5 min, 100°C). All FAME extracts were purified by TLC. The analysis of sample FAME extracts was conducted via GC (GC-17 V3; Shimadzu, Tokyo, Japan) equipped with an autosampler and a flame ionization detector.

Two different GC procedures were required to analyse the FAME distribution of these samples. The first method determined the identity and general fatty acid distribution of fatty acids ranging from four to twenty-five carbon atoms in length, including total CLA, using a fused-silica capillary column DB-225 ms (60 m × 0·25 mm internal diameter, film thickness 0·25 μm; J&W Scientific, Folsom, USA). The second GC method separates the *cis* and *trans* isomers of 18 : 1 fatty acid using a fused-silica capillary column CP-select (200 m × 0·25 mm internal diameter, film thickness 0·25-μm; Varian, Middelburg, The Netherlands). In the first GC analysis, *c*9,*t*11 CLA co-eluted with two minor CLA isomers (*t*8,*c*10 and *t*7,*c*9). The final stage of the FAME analysis was a determination of the distribution of CLA isomers by Ag^+ HPLC (LC10A; Shimadzu). The exact details of the methodologies have been published in Kraft *et al.* (2003). The proportions of separated fatty acid from the lipids in the food, serum and RBCM are expressed as mg/g of total FAME.

Estimation of the conversion rate

The conversion rate of *t*VA to *c*9,*t*11 CLA was estimated according to Turpeinen *et al.* (2002). The individual conversion rate of serum *t*VA for each test-group subject was estimated by the net change in *c*9,*t*11 CLA level (Δ*c*9,*t*11 CLA) compared the sum of the net change in *t*VA level (Δ*t*VA) and Δ*c*9,*t*11 CLA level over the test periods of 7 d (equation 1, comparing 7 d with 0 d) and 42 d (equation 2, comparing 42 d with 0 d), respectively.

Following this, the term Δ*t*VA was the proportion of *t*VA that was not converted and Δ*c*9,*t*11 CLA was the proportion of converted *t*VA, on condition that the subjects received a diet free of CLA and *t*VA. In addition, the slope of the linear regression of Δ*c*9,*t*11 CLA *v*. the sum of Δ*t*VA and Δ*c*9,*t*11 CLA represents the mean conversion (Turpeinen *et al.* 2002). The conversion rate of *t*12 to *c*9,*t*12-18 : 2 was estimated in the same manner.

$$\mathrm{CR} = \frac{\Delta c9,t11\mathrm{CLA}_{7\mathrm{d}}}{\Delta t\mathrm{VA}_{7\mathrm{d}} + c9,t11\mathrm{CLA}_{7\mathrm{d}}} \times 100 = \frac{c9,t11\mathrm{CLA}_{7\mathrm{d}} - c9,t11\mathrm{CLA}_{0\mathrm{d}}}{(t\mathrm{VA}_{7\mathrm{d}} - t\mathrm{VA}_{0\mathrm{d}}) + (c9,t11\mathrm{CLA}_{7\mathrm{d}} - c9,t11\mathrm{CLA}_{0\mathrm{d}})} \times 100 \quad (1)$$

$$\mathrm{CR} = \frac{\Delta c9,t11\mathrm{CLA}_{42\mathrm{d}}}{\Delta t\mathrm{VA}_{42\mathrm{d}} + c9,t11\mathrm{CLA}_{42\mathrm{d}}} \times 100 = \frac{c9,t11\mathrm{CLA}_{42\mathrm{d}} - c9,t11\mathrm{CLA}_{0\mathrm{d}}}{(t\mathrm{VA}_{42\mathrm{d}} - t\mathrm{VA}_{0\mathrm{d}}) + (c9,t11\mathrm{CLA}_{42\mathrm{d}} - c9,t11\mathrm{CLA}_{0\mathrm{d}})} \times 100 \quad (2)$$

Statistical analysis

All statistical analysis were performed using SPSS version 11·5 (SPSS Inc., Chicago, IL, USA) with $P<0{\cdot}05$ taken to indicate significant intra- and intergroup changes. The results are stated as means and standard deviations. Possible differences between the different groups after intervention were analysed with the non-parametric Mann–Whitney *U*-test. Differences between the adaptation period and the intervention period within the treatment groups were analysed

with the Wilcoxon test. Correlations were calculated using Pearson's correlation analysis.

Results

Diet

All participants tolerated the experimental fats well. Subjects showed no change in anthropometric data (body weight, BMI, fat mass, etc.) during the study. The intake in both treatment groups of DM, carbohydrates, protein and dietary fibre during the adaptation and intervention period did not significantly differ (Table 2). The total fat intake of both treatment groups increased after supplementation with the experimental fat. In general, women in both treatment groups showed a lower food intake than men, but the fatty acid composition of their diet did not differ (Table 2). The dietary fatty acid composition of the control group during the intervention was identical to that seen in both study groups in the adaptation period. The fatty acid composition of the test-group diet contained both supplemented *trans* isomers (~8 % of fat intake), which replaced similar proportions of *c*9-18 : 1 and *c*9,*c*12-18 : 2 fatty acids compared with the adaptation diet (Table 2). No difference in total C18 fatty acid intake was observed between the control group and the test group. The standardized diet contained only marginal amounts of *trans*-fatty acids and CLA, as planned (Table 2).

Serum

Despite the fact that blood samples collected after 7 d of the intervention period from the test group were not associated with the standardized diet, the serum fatty acid distribution of these samples showed no significant differences when compared with samples from the test group after 42 d of intervention, with the exception of *t*VA and *t*12 levels (Table 3). The fatty acid distribution of serum lipids did not differ between the men and women in both study groups. No significant differences in total serum C18 fatty acid level were detected between the two study groups, and their total serum C18 fatty acid levels were comparable with those seen during the adaptation period (Table 3). The *t*VA serum level of the test group increased by fivefold and eightfold, whereas the *t*12 serum level increased by ninefold and 12-fold, after 7 d and 42 d of intervention, respectively, compared with the adaptation period ($P \leq 0{\cdot}002$). The serum *c*9,*t*11 CLA level of the test group increased by the 1·7- and 2·0-fold after 7 d and 42 d of intervention, respectively, compared with the adaptation period ($P \leq 0{\cdot}001$). The concentration of serum *c*9,*t*12-18 : 2 remained unchanged in the test group samples. The increase in *t*VA and *t*12 levels after 7 d of *trans*-isomer mixture supplementation ($\Delta t\text{VA} = 0{\cdot}28$, $\Delta t12 = 0{\cdot}56$;% FAME) were greater than the increase from 7 d to 42 d ($\Delta t\text{VA} = 0{\cdot}17$, $\Delta t12 = 0{\cdot}21$;% FAME). The control group

Table 2. Daily intake of macronutrients, *cis*- (*c*) and *trans*- (*t*) isomers of 18 : 1 and *c*9,*t*11 conjugated linoleic acid (CLA) according to duplicate portion analysis of the standardized diet during the adaptation and intervention periods of both groups

(Mean values and standard deviations)

		Adaptation period		Intervention period			
		Total subjects (*n* 24)		Test group (*n* 12)		Control group (*n* 12)	
Intake		Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	M	11·0	1·2	10·5	1·3	11·4	0·6
	W	8·9	1·3	8·7	1·1	8·5	1·9
g/d							
DM	M	589	69	555	81	596	31
	W	479	73	446	129	457	61
Carbohydrates*	M	391	51	371	64	389	25
	W	330	56	298	89	305	47
Protein	M	77	9	73	11	80	3
	W	61	8	58	9	58	8
Total fat	M	78[a]	9	81[b]	8	83[b]	5
	W	60[a]	10	64[b]	12	66[b]	10
Dietary fibre	M	39	5	36	6	38	3
	W	29	5	27	7	28	4
*t*11–18 : 1		0·02[a]	0·00	2·89[b]	0·00	0·02[a]	0·00
*t*12–18 : 1		0·02[a]	0·00	2·91[b]	0·00	0·02[a]	0·00
*c*11–18 : 1		0·99	0·18	0·98	0·17	1·10	0·17
*c*12–18 : 1		0·01[a]	0·00	1·14[b]	0·00	0·01[a]	0·00
*c*9,*t*11 CLA		0·01	0·00	0·01	0·00	0·01	0·00
*c*9,*t*12–18 : 2		0·01	0·00	0·01	0·00	0·01	0·01
% of fat intake							
18 : 0		5·9	0·5	6·4	0·4	5·9	0·6
*c*9–18 : 1		28·7[a]	2·7	24·2[b]	1·8	28·3[a]	1·6
*c*9,*c*12–18 : 2		25·1[a]	3·9	21·0[b]	4·8	25·8[a]	3·1
Σ*Trans*-fatty acids		0·2[a]	0·0	8·5[b]	1·6	0·2[a]	0·0
Σ C18		63·3	6·4	65·1	7·6	63·6	6·2

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

*Calculated as the difference between DM and the content of protein, fat and dietary fibre. Data that were broken down according to gender were significantly different. M, men; W, women.

serum levels of *t*VA, *t*12, and *c*9,*t*11 CLA after 42 d remained unchanged throughout the intervention and were significantly lower that those of the test group ($P \leq 0{\cdot}005$; Table 3).

The slope of the linear regression of $\Delta c9,t11$ CLA *v.* the sum of ΔtVA and $\Delta c9,t11$ CLA in the serum lipids of the test group after 7 d ($P=0{\cdot}001$) and 42 d ($P=0{\cdot}001$) of intervention represents the percentage conversion (Fig. 2). The mean conversion rate of *t*VA after 7 d and 42 d was 24 (SD 10) % and 25 (SD 9) %, respectively. After 7 d of intervention, men showed a lower conversion rate (15 (SD 8) %) than women (31 (SD 6) %; $P=0{\cdot}004$). In contrast, after 42 d of intervention, the conversion rates of both genders were identical (men 23 (SD 6) %, women 26 (SD 11) %; $P=0{\cdot}537$).

After 7 and 42 d, all test-group subjects showed an increase in *t*VA in their serum lipids. Subjects in the test group demonstrated a highly individualistic conversion of *t*VA to *c*9,*t*11 CLA. Thus, the conversion rate of serum *t*VA ranged from 5 % to 37 % (7 d) and from 14 % to 40 % (42 d). Some subjects showed an higher conversion rate initially than after 42 d of intervention, and vice versa. The highest intra-individual range of conversion rate was from 5 % (7 d) to 28 % (42 d). One test-group subject showed no increase in *c*9,*t*11 CLA after 42 d compared with the adaptation period. Thus, no conversion of *t*VA in the serum was verified ('non-responder'). In general, no conversion of *t*12 to *c*9*t*12-18 : 2 was determined in test-group serum samples at both times (7 d, 42 d).

Analysis of the distribution of CLA isomers showed that the major CLA isomer in the serum was *c*9,*t*11 CLA (76 (SD 4) % of total CLA in the adaptation period). During the intervention, serum *c*9,*t*11 CLA levels in the test group increased to 79 (SD 5) % and 84 (SD 5) % of total CLA after 7 d and 42 d, respectively ($P=0{\cdot}136$, $P=0{\cdot}034$). In contrast, after 42 d, the *c*9,*t*11 CLA levels of the control group decreased to 72 (SD 4) % of total CLA ($P=0{\cdot}010$) and were significantly lower than those found in the test group ($P=0{\cdot}002$).

Red blood cell membranes

RBCM lipids were not determined after 7 d of the intervention period because no detectable incorporation of *c*9,*t*11 CLA into the membranes was expected. The fatty acid distribution of RBCM lipids did not differ between men and women in the two groups. In the test group, RBCM *t*VA levels increased significantly by fivefold ($P = 0{\cdot}002$), and *t*12 levels increased

Table 3. The fatty acid distribution of lipids in the serum and red blood cell membranes (RBCM) of the test group and control group during the study (mg/g total fatty acid methyl esters)

(Mean values and standard deviations)

	Adaptation period		Intervention period					
	Total subjects (*n* 24)		Test group (7 d)*		Test group (42 d)*		Control group (42 d)	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fatty acid distribution of serum lipids								
16 : 0	20·24	1·95	21·42	2·36	19·63	3·03	20·05	2·99
16 : 1	2·37	0·83	2·42	1·07	2·31	0·61	2·48	1·06
18 : 0	6·20^{a}	0·45	5·80^{a,b}	0·52	5·07^{a}	0·76	5·76^{b}	0·83
c−18 : 1	18·85	1·54	19·84	2·27	18·55	1·87	18·07	1·50
*t*11−18 : 1	0·07^{a}	0·02	0·35^{b}	0·09	0·52^{c}	0·10	0·07^{a}	0·02
*t*12−18 : 1	0·07^{a}	0·02	0·63^{b}	0·16	0·84^{c}	0·15	0·08^{a}	0·02
*c*11−18 : 1	2·01^{a}	0·24	2·21^{b}	0·27	2·47^{b}	0·28	2·05^{a}	0·23
*c*12−18 : 1	0·04^{a}	0·01	0·41^{b}	0·14	0·47^{b}	0·09	0·07^{a}	0·07
*c*9,*c*12−18 : 2	34·38^{a,b}	3·29	31·65^{a}	2·69	33·60^{a,b}	3·07	36·04^{b}	4·09
*c*9,*t*12−18 : 2	0·01	0·01	0·03	0·02	0·01	0·01	0·01	0·01
*c*9,*t*11 CLA	0·16^{a}	0·04	0·27^{b}	0·10	0·32^{b}	0·10	0·15^{a}	0·06
Σ CLA	0·21^{a}	0·04	0·35^{b}	0·13	0·40^{b}	0·11	0·21^{a}	0·08
20 : 4	7·02^{a}	0·24	6·32^{b}	1·25	6·62^{a,b}	1·48	5·32^{a,b}	2·13
Σ C_{18}	62·95	3·24	63·14	3·58	63·23	7·45	64·13	4·42
Fatty acid distribution of lipids of RBCM								
16 : 0	25·51^{a}	2·35	≠	≠	30·39^{b}	2·77	30·33^{b}	3·41
16 : 1	0·46	0·18	≠	≠	0·43	0·15	0·43	0·24
18 : 0	10·28^{a}	0·88	≠	≠	11·10^{a,b}	1·88	12·12^{b}	3·10
*c*9−18 : 1	16·20^{a}	1·41	≠	≠	19·17^{b}	2·04	18·02^{b}	1·68
*t*11−18 : 1	0·09^{a}	0·01	≠	≠	0·43^{b}	0·06	0·08^{a}	0·02
*t*12−18 : 1	0·10^{a}	0·02	≠	≠	0·87^{b}	0·15	0·11^{a}	0·04
*c*11−18 : 1	1·51^{a}	0·19	≠	≠	2·33^{b}	0·35	1·82^{a}	0·24
*c*12−18 : 1	0·07^{a}	0·02	≠	≠	0·45^{b}	0·24	0·08^{a}	0·07
*c*9,*c*12−18 : 2	14·65	1·44	≠	≠	14·11	1·72	15·40	2·76
*c*9,*t*12−18 : 2	0·07	0·03	≠	≠	0·06	0·02	0·07	0·01
*c*9,*t*11 CLA	0·15^{a}	0·04	≠	≠	0·18^{b}	0·05	0·08^{c}	0·02
Σ CLA	0·19^{a}	0·05	≠	≠	0·21^{b}	0·06	0·11^{c}	0·03
20 : 4	13·82^{a}	0·99	≠	≠	8·66^{b}	3·17	9·22^{b}	3·00
Σ C_{18}	45·94^{a}	2·64	≠	≠	51·02^{b}	2·94	50·30^{b}	1·80

c, *cis*; *t*, *trans*; CLA, conjugated linoleic acid.

^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* Serum lipids in the test group were analysed after 7 d and 42 d of intervention.

≠ Not analysed after 7 d.

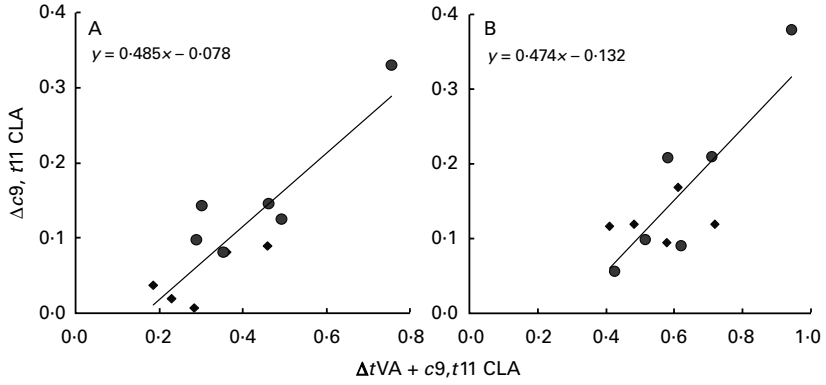

Fig. 2. Linear regression between the net change in *c*9,*t*11 conjugated linoleic acid (CLA (Δ*c*9,*t*11 CLA) and *trans*-vaccenic acid (Δ*t*VA) + Δ*c*9,*t*11 conjugated linoleic acid (CLA) in the serum lipids of the test group after 7 d (A) and 42 d (B) of intervention. The slope represents the average conversion of *t*VA. ●, female subjects; ◆, male subjects.

significantly by ninefold ($P=0{\cdot}002$) after 42 d compared with the adaptation period (Table 3). In addition, the test-group *c*9,*t*11 CLA levels in the RBCM increased significantly from 0·15 % to 0·18 % of total FAME ($P=0{\cdot}021$), whereas no change in *c*9,*t*12-18 : 2 was observed (Table 3). In one test-group subject, the *c*9,*t*11 CLA level in the RBCM decreased by approximately half of its adaptation period value over the 42 d intervention despite a large increase of *t*VA in the RBCM. In addition, the increase in *t*VA in this subject was about 30 % higher than that of the other test-group subjects. This so-called non-responder was excluded from the mean calculations of serum and RBCM lipid analysis in the test group (therefore *n* 11).

After the 42 d intervention period, the control group showed a significant lowering of *c*9,*t*11 CLA by ~50 % (Δ 0·07 % FAME) compared with the adaptation period ($P\leq 0{\cdot}01$), whereas levels of *t*11, *t*12 and *c*9,*t*12-18 : 2 were unchanged (Table 3). Assuming that, without *t*VA supplementation, the *c*9,*t*11 CLA levels of test-group RBCM would be decreased as in control subjects, the mean Δ*c*9,*t*11 CLA of control-group RBCM after 42 d (0·07) was included as a correction factor in equation 2 for each test-group subject (equation 3)

$$\mathrm{CR} = \frac{\Delta c9,t11\mathrm{CLA}_{t\mathrm{group}} + 0{\cdot}07}{\Delta t\mathrm{VA}_{t\mathrm{group}} + (\Delta c9,t11\mathrm{CLA}_{t\mathrm{group}} + 0{\cdot}07)} \times 100 \quad (3)$$

To estimate the average of conversion of *t*VA in the test group, a linear regression was performed, which showed a linear trend ($y = 0{\cdot}234x - 0{\cdot}003$; $P=0{\cdot}066$). The calculated conversion rate of *t*VA to *c*9,*t*11 CLA of the test group was in 19 (SD 3) % (equation 3). The conversion rate estimated from the RBCM ranged from 15 % to 25 %. In these data, the correction factor was, however, included for each test-group subject so the range is not representative.

After the adaptation period, the *c*9,*t*11 isomer represented 78 (SD 4) % of total CLA of RBCM. After 42 d of intervention for the test group, only the *c*9,*t*11 isomer was increased to 83 (SD 7) % of total CLA, whereas in the control group it was reduced to 75 (SD 7) % and was significantly lower than that of the test group ($P=0{\cdot}029$).

Discussion

The Δ9-desaturase – an enzyme that desaturates saturated fatty acid to MUFA (e.g. stearic to oleic acid) – of rat liver microsomes converted *t*VA to *c*9,*t*11 CLA and *t*12 to *c*9,*t*12-18 : 2 (Mahfouz *et al.* 1980; Pollard *et al.* 1980; Holman & Mahfouz, 1981). The present study demonstrated that dietary *t*VA was effectively Δ9-desaturated compared with *t*12. Increased *t*VA concentrations in serum as well as in RBCM were associated with increased *c*9,*t*11 CLA concentrations in serum and RBCM (Table 3).

Previous studies in animals observed the conversion of dietary *t*VA to CLA and its accumulation in different body tissues (Ip *et al.* 1999; Gläser *et al.* 2000; Santora *et al.* 2000; Banni *et al.* 2001; Loor *et al.* 2002; Kraft, 2004). Furthermore, studies in humans have also described an increase in *c*9,*t*11 CLA levels when *t*VA was supplemented (Salminen *et al.* 1998; Adlof *et al.* 2000; Turpeinen *et al.* 2002; Table 4).

Turpeinen *et al.* (2002) used the same fatty acid preparation as in previous studies with different dosages (1·5 g, 3·0 g and 4·5 g *t*VA/d, respectively) during a 9 d trial period. We conducted a study over the longer period of 42 d to determine the conversion rate after a long-term intervention and to investigate the incorporation of supplemented *trans*-18 : 1 isomers into tissues such as RBCM. Furthermore, to in order estimate the conversion rate after short-term intervention and compare the results with those of Turpeinen *et al.* (2002), blood samples were collected after 7 d.

Turpeinen *et al.* (2002) observed similar short-term results, producing a 307 % increase in serum *t*VA level from a dietary intake of 3·0 g *t*VA/d (corresponding value in the present study, 400 %; Table 3). At a dosage of 4·5 g *t*VA/d, serum *t*VA increased after 9 d by about 620 %, which is similar to the value seen when 3·0 g dietary *t*VA/d was given over a 42 d period (643 %, Table 3). The increase in serum *t*VA was related with an increase in *c*9,*t*11 CLA in the serum lipids in both this and Turpeinen's studies (Fig. 2; see also Turpeinen *et al.* 2002). The conversion rate of serum *t*VA determined by Turpeinen *et al.* (2002) was on average 19 %. In our preliminary study (unpublished results; study conducted under the same conditions) with women who consumed 1·2 g *t*VA daily over 28 d,

Table 4. Studies concerning the conversion of *trans*-vaccenic acid (*t*VA) to conjugated linoleic acid (CLA) in man

				Own results	
	Salminen *et al.* 1998	Adlof *et al.* 2000	Turpeinen *et al.* 2002	Unpublished data‡	Present study
Subjects	49 ♂, 31 ♀	1 ♂	8 ♂, 22 ♀	12 ♀	12 ♂, 12 ♀
Duration (d)	40	2	9	28	42
Dose (g/d)	high-TFA diet, ~3·0 *t*VA	8 *t*VA†	1·5, 3·0, 4·5 *t*VA	1·2 *t*VA	3·0 *t*VA
*c*9,*t*11 CLA* (mg/g fatty acid methyl esters)	0·43	0·32	0·24, 0·35, 0·44	0·36	0·27§, 0·32ψ
Increase of CLA (%)	30	257	50, 169, 193	76	69§, 100ψ
Conversion rate (%)	–	–	19	20	24§, 25ψ

* In serum.
† Single dose of deuterium-labelled *t*VA.
‡ Under the same conditions as in the present study.
§ 7 d of intervention.
ψ 42 d of intervention.

elevated serum *c*9,*t*11 CLA levels were observed as well. The conversion rate was also about 20 % (Table 4). The conversion rates obtained in these studies are consistent with that obtained (25 %) after 42 d with 3·0 g *t*VA (Table 4).

This calculation of the conversion rate was only an estimation approximately how much of the supplemented *t*VA and *t*12 was converted to *c*9,*t*11 CLA and *c*9,*t*12-18 : 2, respectively by Δ9-desaturation. This calculation is only a net end-product estimation. The ratio of the change in *t*VA and *c*9,*t*11 CLA, and in *t*12 and *c*9,*t*12-18 : 2, respectively, relative to the adaptation period do not reflect their real gross conversion rate but the net sum of their surviving products. These estimates of conversion rate are influenced by several metabolic processes (e.g. ß-oxidation, elongation) and by incorporation into the specific tissue lipids. It is difficult to incorporate the oxidation rates of the supplemented fatty acids and their desaturation products to the calculation of conversion in humans. Sergiel *et al.* (2001) showed, in rats, that *c*9,*t*11 CLA was oxidized significantly more than linoleic acid. Thus, the real levels of *c*9,*t*11 CLA synthesized from *t*VA are probably higher.

It appears that CLA formed by the endogenous desaturation of *t*VA is incorporated primarily into neutral lipids and secondarily into various classes of phospholipids (Banni *et al.* 2001). The increase in *c*9,*t*11 CLA in the RBCM was greater than that of *t*10,*c*12 CLA, suggesting that the extent of incorporation of individual CLA isomers may be tissue dependent (Burdge *et al.* 2005). Kraft (2004) showed the highest accumulation of endogenously synthesized CLA in tissues rich in neutral lipids, for example, adipose tissue, followed by the gonads, thymus, kidney, muscle, liver, etc. The conversion rate thus differed between different pools and organs (e.g. serum 22 %, muscle 20 %, liver 17 %; Kraft, 2004) and might depend on the content of phospholipids and neutral lipids and on tissue-specific metabolic rates, that of heart, for example, being 8 %.

Serum levels reflect only the dietary intake of the previous few days (Kohlmeier, 1995). RBCM provide a marker reflecting a longer-term intake and offer a more aggregated time period than serum (Arab, 2003). Human RBC have a mean lifetime of about 120 d (Loeffler, 2005) and their membranes reflect the intake over this lifespan (Arab, 2003). We therefore used the RBCM as a low-invasion method to analyse the incorporation of fatty acids during this study, with the assumption that approximately one third of RBC were renewed after the intervention period (42 d).

The RBCM of pigs fed CLA (Stangl *et al.* 1999) and of rats fed *t*VA (Kraft, 2004) showed a linear increase in *c*9,*t*11 CLA. Obviously, dietary *t*VA and *t*12 as well as endogenously synthesized *c*9,*t*11 CLA were incorporated into test-group RBCM after 42 d (Table 3). Fatty acid analysis of the RBCM revealed a decrease in *c*9,*t*11 CLA in the control group (Table 3). These results indicated clearly that the diet supplied was poor in *trans*-fatty acids and CLA, and that these subjects had complied with the required study diet. In addition, this decrease in *c*9,*t*11 CLA could be included in calculations of the conversion rate of incorporated fatty acids and was able to produce an improved estimation of the *t*VA conversion rate, which was 19 (SD 3) %. The relation between the mean content of *t*VA and *c*9,*t*11 CLA in the test-group RBCM after 42 d and that in the control group after 42 d resulted in a *t*VA conversion rate of approximately 23 %. Both methods corroborate the *t*VA conversion rate estimated using serum.

Furthermore, the conversion rate in rats calculated by the net changes in serum *t*VA and CLA was 22 %, which was nearly equivalent to the whole-body conversion rate in the rat (25 %, mean of all tissue conversion rates; Kraft, 2004). These results suggest that the *t*VA conversion rate estimated by human serum is also representative of that for the whole human body.

In general, the conversion rate of *t*VA estimated from the serum showed a wide range within subjects and between times of intervention (7 d, 42 d). Turpeinen *et al.* (2002) also found interindividual differences in the conversion rate of serum *t*VA (e.g. non-responder, low-responder). Furthermore, after 7 d of intervention in the present study, women showed a higher *t*VA conversion rate than men, whereas after 42 d no difference was observed.

First, the diet at the time of blood collection at day 7 was not the standardized diet and was not similarly controlled. The differences in conversion rate between 7 d and 42 d of intervention (gender specific and intra-individual) could be partly explained by dietary factors in the individual diets. Dietary factors such as cholesterol, PUFA, carbohydrates and vitamin A have been shown to affect the Δ9-desaturase activity in mammals (Ntambi, 1992; Miller *et al.* 1997; Sessler & Ntambi, 1998; Tocher *et al.* 1998; Ntambi, 1999; Ntambi, 2004). However, the desaturation indices (18 : 1/18 : 0, 16 : 1/16 : 0), which are related to the Δ9-desaturase activity (Lee *et al.* 1996; Santora *et al.* 2000; Pala *et al.* 2001), did not

differ between treatment groups, genders and blood collection times (0 d, 7 d, 42 d; data not shown).

Second, it is conceivable that there are gene polymorphisms of Δ9-desaturase (also described for other enzymes of lipid metabolism; e.g., Halsall *et al.* 2000; Galluzzi *et al.* 2001; Talmud *et al.* 2001) that may determine gene expression, enzyme activity or substrate specificity. The conversion rate estimated from the serum could be more dependent on individual differences in fatty acid metabolism (differences in enzyme activities and saturations, abundance of cellular signalling transduction elements and substrate kinetics; Mittendorfer *et al.* 2005). These facts might also explain the observed interindividual differences in conversion rate and the findings in the non-responder. In addition, gender-specific differences in the expression of Δ9-desaturase were observed in mice (Lee *et al.* 1996) and could be partly related to hormones (Tocher *et al.* 1998; Miyazaki *et al.* 2003; Cohen & Friedman, 2004) or to body fat mass (Legrand & Hermier, 1992; Jones *et al.* 1996).

In the present study, the non-responder (with no conversion of the supplemented *t*VA) identified on RBCM analysis was excluded from the data analysis. The inclusion of this subject's data in the conversion rate calculation does not seriously change the mean conversion rates (serum 24 (SD 10) %, 7 d, 23 (SD 11) %, 42 d; RBCM 18 (SD 6) %).

Altogether, serum lipids are adequate to estimate the conversion rate of *t*VA, especially after a short-term intervention, whereas RBCM lipids were better for estimating the conversion rate of *t*VA into incorporated fatty acid after a longer intervention period. The conversion rates estimated by serum and RBCM were similar and ranged on average from 19 % to 25 %.

Despite an increase in *t*12 in lipids in the serum and RBCM of the test group, no significant increase in *c*9,*t*12-18 : 2 concentration was observed (Table 3). Thus, no conversion of supplemented *t*12 could be assessed via serum and RBCM samples. In other studies, Salminen *et al.* (1998) did not analyse any individual *trans*-18 : 1 isomers, and Turpeinen *et al.* (2002) gave no detailed information on serum *t*12 and *c*9,*t*12-18 : 2 levels. In cows receiving an abomasal infusion of a mixture of *t*VA and *t*12, these fatty acids and their desaturation products *c*9,*t*11 CLA and *c*9,*t*12-18 : 2 were incorporated into milk fat (Griinari *et al.* 2000). The increases in *t*12 and *c*9,*t*12-18 : 2 level (64 %) were higher than the increases in *t*VA and *c*9,*t*11 CLA level (40 %), whereas a higher conversion rate was observed for *t*VA (31 %) than *t*12 (10 %; Griinari *et al.* 2000). In rats fed *t*VA and *t*12, the conversion rate of *t*VA was also substantially higher than the conversion rate of *t*12 (Kraft, 2004). In the present study, the mean increase in *t*12 level was generally 30 % higher than the mean increase in *t*VA level (including ~22 % converted *t*VA) in both serum and RBCM. The greater increase in *t*12 levels is approved by the literature (Griinari *et al*. 2000; Kraft, 2004), and, in general, *t*VA, compared with *t*12, is preferentially metabolized by desaturation, especially Δ9, elongation and β-oxidation. Furthermore, there are different rates of activation to their CoA-esters before desaturation (Lippel 1973).

The consumption of *trans*-fatty acids and their effects on human health is still under review (European Food Safety Authority, 2004; Weggemans *et al.* 2004, Lock *et al.* 2005). The most important factor to consider when comparing *t*VA with other *trans*-18 : 1 isomers is that *t*VA is readily converted to *c*9,*t*11 CLA. Further research is required into the mechanisms of *t*VA desaturation and the effects of individual *trans*-18 : 1 isomers on human health.

We can conclude from the present study that *t*VA was effectively Δ9-desaturated to *c*9,*t*11 CLA, whereas a conversion of *t*12 to *c*9,*t*12-18 : 2 could not be detected. The average conversion rate of *t*VA in serum was 24 %, and the value from fatty acids incorporated into RBCM was 19 %. The conversion of *t*VA to *c*9,*t*11 CLA (20–25 %) should be taken into account in future studies when determining the CLA supply.

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CHAPTER 3

Dietary supplementation with 11*trans*- and 12*trans*-18:1 and oxidative stress in humans.

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Dietary supplementation with 11*trans*- and 12*trans*-18:1 and oxidative stress in humans[1–3]

Katrin Kuhnt, Andreas Wagner, Jana Kraft, Samar Basu, and Gerhard Jahreis

ABSTRACT

Background: High consumption of *trans* fat has been associated with high oxidative stress in humans, which could increase the risk of the development or acceleration of several diseases, such as atherosclerosis, cancer, and type 2 diabetes.

Objective: Several urinary and blood biomarkers of oxidative stress [8-iso-prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$), 15-keto-dihydro-$PGF_{2\alpha}$, and 7,8-dihydro-8-oxo-2′-deoxy-guanosine in urine and α-,β-,γ-,δ-tocopherol, and retinol in plasma] were monitored to evaluate the oxidative stress induced by dietary supplementation of 11*trans*- and 12*trans*-18:1 isomers in humans during a 6-wk intervention.

Design: After a 14-d adaptation period free of *trans* fatty acid supplementation (baseline), the test group ($n = 12$) received 3.0 g 11*trans*-18:1/d and 3.0 g 12*trans*-18:1/d (Σ 6.0 g/d), and the control group ($n = 12$) consumed a control oil free of *trans* fatty acids and conjugated linoleic acids for 6 wk.

Results: The postintervention concentration of urinary 8-iso-$PGF_{2\alpha}$ (free radical–induced lipid peroxidation) in the test group was significantly higher than baseline and significantly higher than that observed in the control group. The concentrations of 15-keto-dihydro-$PGF_{2\alpha}$ (cyclooxygenase-mediated inflammatory response indicator) and 7,8-dihydro-8-oxo-2′-deoxy-guanosine (oxidative DNA damage) were not affected by the 11*trans*- and 12*trans*-18:1 supplementation.

Conclusions: Although an increase in urinary 8-iso-$PGF_{2\alpha}$ was observed and the effects of prolonged high (ie, >5.0 g/d) consumption of *trans* fat could be relevant to the development of disease, the mean intakes of 11*trans*- and 12*trans*-18:1 in Europeans are estimated to be significantly below the amounts administered in this study (ie, 6.0 g/d); such low intakes could minimize the possible risk of detrimental effects on human health. *Am J Clin Nutr* 2006; 84:981–8.

KEY WORDS Oxidative stress, *trans* fatty acids, conjugated linoleic acids, isoprostanes, prostaglandins, 7,8-dihydro-8-oxo-2′-deoxyguanosine

INTRODUCTION

Oxidative stress is a term commonly used to describe the steady state level of oxidative damage in a cell, tissue, or organ that is caused by the reactive oxygen species, such as free radicals and peroxides, within a biological organism. Oxidative stress is the result of an imbalance between prooxidant and antioxidant processes within that organism in favor of the former. In fact, a greater consumption of *trans* fat has been associated with higher levels of oxidative stress in humans (1–5), and prolonged exposure to high levels of oxidative stress has been implicated in the development or acceleration of several dysfunctions and diseases, such as cardiovascular disease (6–11), inflammation (12, 13), type 2 diabetes (14), and breast, colon, and prostate cancers (15, 16).

The *trans* fats are a class of unsaturated fatty acids that possess at least one double bond in *trans* configuration. Generally, these *trans* fatty acids occur naturally in ruminant fats formed by the enzymatic hydrogenation of several polyunsaturated fatty acids (eg, linoleic acid) in the rumen. They are also formed during industrial processes such as the hydrogenation of vegetable oils. Although ruminant fats and partially hydrogenated vegetable oils (PHVOs) contain the same *trans* fatty acid isomers, their isomeric profiles are clearly different; that is, ruminant fats have the 11*trans*-18:1 (11*t*-18:1; vaccenic acid), which is 60–80% of total 18:1 (17), and PHVOs mainly have the 9*t*-18:1 (elaidic acid) (18). During hydrogenation by rumen bacteria, numerous geometric (*t*,*t*, *c*,*t*, *t*,*c*, and, *c*,*c*) and positional (2,4 to 14,16) isomers of conjugated linoleic acids (CLAs) are created, but 9*cis*,11*trans*-18:2 (9*c*,11*t*-CLA) is the predominant isomer formed (19). In addition, the major source of 9*c*,11*t*-CLA in milk fat is the created via endogenous synthesis of *t*11-18:1 by Δ9-desaturase (20). The endogenous synthesis of 9*c*,11*t*-CLA has also been found in humans (21, 22). The intakes of *trans* fatty acids and their effects on human health are still under review (23). Estimates for dietary intake of *trans* fatty acids differ from country to country. In European countries, the intake of *trans* fatty acids varies from 2.0 to 2.7 g/d (60% from ruminant fats and 40% from PHVOs; 24), whereas the intakes of 11*t*-18:1 range from 0.7 to 1.0 g/d (25) and those of 9*c*,11*t*-CLA range from 0.3 to 0.5 g/d (26). For comparison, in US and Canadian populations, the mean *trans* fatty acid intake was estimated to be higher, ie, 5.8 g/d (27, 28), and, in Canadian women, it was exceedingly high, ie, 10.6 g/d (29). In the US and Canadian populations, ≈80% of

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total *trans* fatty acids is currently derived from PHVOs (28). However, to date, very few human intervention studies have been carried out to evaluate the specific effects of the individual *trans* isomers of 18:1.

At present, the measurement of F_2-isoprostanes is regarded as the gold standard by which to evaluate the level of oxidative stress in vivo; 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is the major F_2-isoprostane successfully evaluated in many experimental and clinical studies (30). It is derived from arachidonic acid by using nonenzymatic free radical–induced peroxidation (30–32). The 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (15-ketodihydro-$PGF_{2\alpha}$) can be used as an indicator for lipid peroxidation through the cyclooxygenase pathway (33). The 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG), a sensitive biomarker monitoring in vivo DNA damage, is eliminated via DNA repair mechanisms and excreted in urine (1, 34). Tocopherols and retinol are measured to assess the status of individual antioxidants (35).

Several studies have shown the association of the effects of the consumption of *trans* fat with higher levels of oxidative stress. In a study with mice fed a *trans* diet (13.6% of energy as fat), an increased plasma concentration of F_2-isoprostanes was found (36). Increases in the concentrations of urinary 8-iso-$PGF_{2\alpha}$ were observed in several studies on the effects of supplementation with 11*t*-18:1, linoleic acid, or CLAs in humans (5, 21, 37–39). In another study, the oxidative DNA damage in women was higher after supplementation with a diet rich in linoleic acid (40). To evaluate oxidative stress during the dietary intake of the 2 *trans* 18:1 isomers (11*t*- and 12*t*-18:1; a total of 6 g/d) for a 6-wk intervention period, several biomarkers of oxidative stress were measured—8-iso-$PGF_{2\alpha}$, 15-ketodihydro-$PGF_{2\alpha}$, and 8-oxodG in urine and α-, β-, γ-, δ-tocopherol, and retinol in plasma.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy subjects (12 women and 12 men) were recruited. The volunteers were informed of the purpose, course, and possible risks of the study. Subjects had no diagnosed diseases, were not taking any medications (eg, aspirin), were not abusers of alcohol, and were not taking any dietary supplements. The volunteers had a mean ± SD age of 24 ± 3 y (range: 20–28 y) and a normal weight with a mean body mass index (in kg/m^2) of 21 ± 2 (range: 19–26). The treatment groups did not differ significantly in anthropometric data (22). Each study group consisted of 6 men and 6 women.

All subjects gave informed written consent. The study was approved by the ethics committee of the Friedrich Schiller University (Jena, Germany).

Diets

Throughout the entire study (8 wk), the diet consumed contained only traces of *trans* fatty acids and CLA, because the food supplied in this study was so chosen to minimize the extraneous amounts of these fatty acids. All volunteers were subjected to a 14-d adaptation period without supplementation to establish baseline conditions for the *trans* fatty acids and CLA concentrations. During the 42-d intervention period, the diet of the test group ($n = 12$) contained no CLA and was supplemented with 3.0 g 11*t*-18:1/d and 3.0 g 12*t*-18:1/d (a total of 6 g/d; *trans*-isomer mixture; Natural ASA, Hovdebygda, Norway; 22). The diet of the control group ($n = 12$) was supplemented with a control oil (palm kernel oil and rapeseed oil, 1:1). The control oil was free of *trans* fatty acids and CLA.

During the adaptation period, all volunteers consumed 20 g of a pure (ie, without supplements), commercially available chocolate spread (Nutella; Ferrero, Frankfurt am Main, Germany) to make the diets isocaloric. For the intervention period, control oil and the *trans*-isomer mixture were added to the chocolate spread to achieve good acceptability. During the intervention period, each subject consumed daily a total of 20 g of the corresponding combined supplement and chocolate spread mixture.

In the last 7 d of each study period, the volunteers were given a standardized diet (**Table 1**). During this time, the food residues were returned and weighed each day to allow for more accurate measurements of food consumption. Duplicate portions of the dietary supplies were collected, freeze-dried, homogenized, and sampled for the nutritional analysis of the study diet. The chemical analyses of the study diet and volunteer's blood samples were conducted by using previously described procedures (22).

Blood and urine sampling

Blood samples were collected on the last day of the standardized diet during the adaptation period (0 d–baseline) and the 42-d intervention period. Blood samples were drawn between 0730 and 0830 by venipuncture into evacuated tubes (BD Vacutainer Systems, Heidelberg, Germany) containing EDTA as an anticoagulant for plasma preparation after overnight fasting.

Urine samples were collected during the last 5 d of the standardized diet of each period. The morning urine on the first collection day was not kept, but urinations during the rest of the day up to the morning urine on the next day (ie, 24-h urine collection) were collected in a special 24-h urine-collection tank (Sarstedt, Nümbrecht, Germany). One percent (by vol) of each 24-h urine was taken with urine-monovettes (Sarstedt) and stored in a frozen state at −80 °C until analysis. In a previous study, Helmerrson and Basu (41) found no significant difference between 8-iso-$PGF_{2\alpha}$ concentrations in urine taken from 24-h samples from different days. However, to minimize the variation in each subject, in the current study, 1% (by vol) of each 24 h-urine sample from 5 consecutive collection days of each subject was pooled before analysis.

Biomarkers of lipid peroxidation (nonenzymatic and enzymatic)

The concentrations of free 8-iso-$PGF_{2\alpha}$ in urine samples were analyzed without extraction by using a highly specific and sensitive radioimmunoassay as previously described (42). Urinary samples were analyzed for 15-ketodihydro-$PGF_{2\alpha}$ without any extraction by using a radioimmunoassay as described previously (33).

The urinary concentrations of 15-ketodihydro-$PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$ were adjusted by creatinine values to correct for variations in the glomerular filtration rate. Urinary creatinine concentrations were determined by using a commercial kit (IL Test; Monarch Instruments, Amherst, MA).

Measurement of urinary 7,8-dihydro-8-oxo-2′-deoxyguanosine

Urine samples were acidified by using acetic acid (pH 6.5). After centrifugation (4000 U/min; 1800 × *g* at 21 °C for 15 min),

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TABLE 1
Nutritional evaluation of daily dietary intakes in the control and test groups during the adaptation and intervention periods of the dietary supplementation study[1]

	Adaptation period		Intervention period		*P* for treatment effects[2]
Intake	Control group	Test group	Control group	Test group	
Energy (MJ)	10.0 ± 2.1[3]	10.0 ± 2.0	10.1 ± 1.6	9.7 ± 1.6	NS
Carbohydrates (% of energy)[4]	61.3 ± 3.4	63.0 ± 3.3	59.4 ± 2.6	59.9 ± 3.8	NS
Protein (% of energy)	12.3 ± 0.9	12.3 ± 0.5	12.1 ± 0.8	12.2 ± 0.8	NS
Total fat (% of energy)	28.0 ± 2.7	26.2 ± 3.3	30.0 ± 2.1	29.4 ± 3.8	NS
SFA (% of fat)	25.1 ± 2.7	26.1 ± 2.4	26.2 ± 2.5	25.2 ± 2.6	NS
MUFA (% of fat)[5]	33.0 ± 1.8	32.5 ± 2.8	33.1 ± 1.9	31.2 ± 2.5	<0.001
PUFA (% of fat)	28.2 ± 3.5	27.6 ± 4.3	28.6 ± 3.0	23.6 ± 4.4	<0.001
TFA (% of fat)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	8.5 ± 0.1	<0.001
TE (mg)[6]	15.1 ± 3.5	15.2 ± 2.5	14.9 ± 3.5	14.6 ± 2.6	NS
α-Tocopherol (mg)	14.9 ± 3.4	15.3 ± 2.4	14.6 ± 3.5	14.3 ± 2.5	NS
γ-Tocopherol (mg)	2.2 ± 0.6	2.1 ± 0.4	2.7 ± 0.4	2.4 ± 0.4	<0.001
Retinol (mg)	2.3 ± 0.7	1.9 ± 0.5	2.2 ± 0.6	1.7 ± 0.6	NS
Daily portion from supplement					
α-Tocopherol (mg)	1.14[7]	1.14	1.16	1.10	
γ-Tocopherol (mg)	0.87	0.87	0.94	1.09	
Retinol (mg)	0.36	0.36	0.39	0.34	

[1] $n = 12$; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids; TE, tocopherol equivalents. No significant treatment × sex interactions were observed.
[2] Significantly different from the control group with baseline value as the covariate.
[3] $\bar{x}$ ± SD (all such values).
[4] Calculated as the difference between total energy intake and energy intakes from protein, fat, and dietary fiber.
[5] MUFAs with *trans* configuration were not included.
[6] 1 mg TE = 1 mg α-tocopherol = 10 mg γ-tocopherol = 1.49 IU.
[7] $\bar{x}$ (all such values).

urine samples were purified by using solid-phase extraction with C18 EC columns (Macherey-Nagel, Dueren, Germany). The analysis of 8-oxodG was conducted with the use of HPLC (column: Hypersil C18 ODS II, 5 μm, 250 × 4 mm; Agilent, Waldbronn, Germany) by using a gradient elution of sodium citrate (pH 5) as solvent A and acetonitrile as solvent B at a flow rate of 1 mL/min. The detailed gradient profile was as follows: isocratic elution with 2% solvent B for 10 min, a gradient of 10% solvent B for 5 min, further elution with 10% solvent B for 3 min, and reequilibration with 2% solvent B for 5 min. The detection of 8-oxodG was followed by electrochemical detection (0.550 V) and diode-array detection at different wave lengths (254, 260, and 280 nm). Standards (8-bromoguanosin, isocytosin, and 8-oxodG) were purchased from Sigma-Aldrich (Munich, Germany).

Measurement of plasma tocopherols and retinol

Retinol and tocopherols behave as antioxidants in lipid peroxidation in biological systems (43). Plasma concentrations of α-, β-, γ-, and δ-tocopherol and retinol were analyzed by using HPLC. Plasma (250 μL) was extracted with 1 mL *n*-hexane containing 0.045% of 2.6-Di-*tert*-butyl-*p*-kresol and 250 μL ethanol. After centrifugation (2500 U/min, at 700 × *g* at 21 °C for 5 min), a 20-μL volume of the supernatant solution was used for injection. Tocopherols and retinol were separated by using a Shimadzu 10A series HPLC with a 250 × 4-mm, 5-μm Nucleosil–100 NH_2 column (Macherey-Nagel) by isocratic elution with a ratio of *n*-hexane to 2-propanol (96:4 by vol) at a flow rate of 0.8 mL/min. The tocopherols and retinol were detected by using an RF 10AXL fluorescence detector (excitation wave length 295 nm, emission wave length 335 nm) and diode-array detector (325 nm), respectively. The tocopherol (α, β, γ, δ) and *all-trans*-retinol standards were purchased from Calbiochem (Merck Biosciences, Nottingham, United Kingdom).

Measurement of tocopherols and retinol in supplements and food

Retinol and α- and γ- tocopherol were measured in food and supplements (*trans*-isomer mixture and control oil). The β- and δ-tocopherol concentrations were below the limit of quantification (0.0001 ng/μL). Lyophilized and homogenized food samples from duplicate portions of the standardized diet (1 g) were mixed with 1 g ascorbic acid, saponified by using a ratio of potassium hydroxide to distilled water to ethanol (12:20:100 wt by vol), heated for 40 min at 80 °C, extracted with the use of *n*-hexane in the presence of 0.045% 2.6-Di-*tert*-butyl-*p*-kresol, and washed with 2 mL distilled water. The analyses were based on conditions similar to those described above. The tocopherol and retinol content in food was calculated by using the consumed amount of each food item (wet wt) during the standardized diet to ascertain the total intakes of tocopherols and retinol.

Statistical analysis

All statistical analyses were performed by using SAS software (version 9.1; SAS Institute Inc, Cary, NC). $P \leq 0.05$ was regarded as significant. The data values are stated as means ± SDs. The Kolmogorov-Smirnov test was used to test the distribution of the data, and all measures were normally distributed. The 2-factor analysis of variance was used to compare the data of the 2 treatments. The covariate value was the measurement from day

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TABLE 2
Blood and urinary biomarker concentrations in the control and test groups during the adaptation and intervention periods of dietary supplementation study[1]

Biomarker	Adaptation period		Intervention period		*P* for treatment effect[2]
	Control group	Test group	Control group	Test group	
Plasma					
α-Tocopherol (μmol/L)	24.00 ± 3.37[3]	23.43 ± 4.13	22.46 ± 2.21	22.21 ± 4.55	NS
β-Tocopherol (μmol/L)	0.45 ± 0.18	0.53 ± 0.21	0.54 ± 0.20	0.46 ± 0.19	0.018
γ-Tocopherol (μmol/L)	1.00 ± 0.37	1.09 ± 0.47	1.00 ± 0.30	1.22 ± 0.47	NS
Retinol (μmol/L)	2.19 ± 0.61	2.18 ± 0.39	1.81 ± 0.30	2.07 ± 0.35	0.027
*c*9,*t*11 CLA serum (% of FAME)	0.16 ± 0.04	0.16 ± 0.03	0.15 ± 0.06	0.31 ± 0.08	<0.001
*c*9,*t*11 CLA RBC membranes (% of FAME)	0.14 ± 0.04	0.15 ± 0.04	0.08 ± 0.02	0.18 ± 0.05	<0.001
Urine					
8-iso-$PGF_{2\alpha}$ (nmol/mmol Cr)	0.37 ± 0.09	0.45 ± 0.19	0.38 ± 0.12	0.54 ± 0.25	0.042
15-kd-$PGF_{2\alpha}$ (nmol/mmol Cr)	0.18 ± 0.04	0.23 ± 0.11	0.20 ± 0.06	0.22 ± 0.08	NS
8-oxodG (nmol/mmol Cr)	1.12 ± 0.63	0.92 ± 0.57	1.02 ± 0.51	0.95 ± 0.67	NS

[1] $n = 12$. CLA, conjugated linoleic acid; RBC, red blood cell; FAME, fatty acid methyl ester; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; 15-kd-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$; 8-oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine. No significant treatment × sex interactions were observed.
[2] Significantly different from the control group with baseline value as the covariate.
[3] $\bar{x}$ ± SD (all such values).

0 (baseline) of the study. The treatment × sex interaction was not significant. Correlations were calculated by using the Pearson correlation analysis. Correlation factors were compared by using Fisher's *z*-transformation (*z* test).

RESULTS

All subjects successfully completed the study, and all measured variables were within their normal physiologic range.

Dietary intake

Male subjects tended to have higher daily intakes of food than did the female subjects (baseline: total men 11.0 ± 1.2 MJ/d; total women 8.9 ± 1.7 MJ/d). Nevertheless, the distribution (by % of energy) of carbohydrates, protein, and total fat did not differ between the treatment groups (Table 1). The intakes of monounsaturated fatty acids (*trans* 18:1 was not included) and polyunsaturated fatty acids in the test group were significantly lower than those in the control group, which corresponded to the increase in *trans* fatty acids (11*t*- and 12*t*-18:1) in the test group diet (Table 1).

The intakes of tocopherol equivalents and retinol did not differ significantly between the treatment groups (Table 1). The γ-tocopherol intake during the intervention period was greater in the control group than in the test group. The portions of retinol, α-, and γ-tocopherol in the daily consumed dose of chocolate spread during the adaptation period and the portions received with control oil or the *trans*-isomer mixture during the intervention period did not differ significantly (Table 1).

Plasma tocopherol and retinol concentrations

The mean concentration of plasma δ-tocopherol was 0.001 μmol/L (data not shown). The α- and γ-tocopherol concentrations in plasma remained unchanged after the intervention. The β-tocopherol concentrations in the control group were significantly higher than those in the test group (**Table 2**). Unfortunately, the β-tocopherol concentration of the diet was not measured. The retinol concentration in the control group was significantly lower than that in the test group, although the groups' retinol intakes did not differ significantly (Tables 1 and 2).

9*cis*,11*trans*–conjugated linoleic acid of serum and red blood cell (RBC) membranes

The combined serum and RBC membrane 9*c*,11*t*-CLA concentrations in the test group during the intervention period were significantly higher than those in the test group during baseline or in the control group during the intervention period. In contrast, the combined serum and RBC membrane 9*c*,11*t*-CLA concentrations in the control group decreased from the adaptation to the intervention (Table 2). The 9*c*,11*t*-CLA content of the RBC membranes in the intervention period differed between male and female subjects (control group: 0.06 ± 0.02 in males, 0.09 ± 0.02 in females; $P = 0.022$; test group: 0.16 ± 0.03 in males, 0.21 ± 0.05 in females; $P = 0.084$).

Urinary concentrations

The urinary 8-iso-$PGF_{2\alpha}$ excretion of the test group was significantly greater than the baseline concentration. In contrast, the control group's 8-iso-$PGF_{2\alpha}$ excretion remained constant throughout the study. The test group's 8-iso-$PGF_{2\alpha}$ concentrations after the intervention were significantly higher than those of the control group (Table 2).

The concentrations of urinary 15-ketodihydro-$PGF_{2\alpha}$ detected in both treatment groups' samples were constant throughout the study. No significant differences between the treatment groups or sexes were observed (Table 2).

The comparison between the control and test groups' baseline urinary 8-oxodG concentrations found no significant differences. Overall, the intervention period urinary concentration of 8-oxodG remained constant for both treatment groups throughout the study (Table 2).

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TABLE 3
Correlation coefficients of the urinary biomarker concentrations of control and test groups during a dietary supplementation study[1]

			P[3]		
	Correlation coefficient[2]			Between group	
Biomarker and group	Adaptation	Intervention	Within group	Adaptation	Intervention
Correlation with 8-iso-$PGF_{2\alpha}$					
15-kd-$PGF_{2\alpha}$					0.116
Control	0.597[4]	0.636[4]	0.894		
Test	0.866[4]	0.011	0.005	0.183	
8-oxodG					0.857
Control	−0.494	−0.054	0.301		
Test	−0.012	0.031	0.927	0.261	
Correlation with 15-kd-$PGF_{2\alpha}$					
8-oxodG					0.420
Control	−0.426	−0.122	0.481		
Test	0.090	0.252	0.722	0.247	

[1] 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; 15-kd-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$; 8-oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine.
[2] Calculated by using Pearson correlation analysis ($n = 12$).
[3] The z test method. P values for within- and between-group differences are shown in the table ($n = 12$).
[4] $P \leq 0.05$.

Correlation analysis

During the adaptation period, urinary 8-iso-$PGF_{2\alpha}$ concentrations correlated significantly with urinary 15-ketodihydro-$PGF_{2\alpha}$ concentrations in the control and test groups (**Table 3**). It is interesting that the comparison of postintervention and baseline urinary 8-iso-$PGF_{2\alpha}$ concentrations found a significantly lower correlation coefficient ($r = 0.011$) in the test group, whereas the correlation coefficient of the control group was unchanged. The urinary 8-oxodG concentration did not correlate with the urinary 8-iso-$PGF_{2\alpha}$ or 15-ketodihydro-$PGF_{2\alpha}$ concentration (Table 3).

In both groups and both study periods, no correlations were found between urinary 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$, respectively, and plasma tocopherols and retinol, respectively (data not shown). Moreover, no correlation between the intakes of tocopherols and retinol, their plasma concentrations, and urinary 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$ concentration was observed.

DISCUSSION

Healthy rats fed a diet containing 0.5% and 1% 11*t*- and 12*t*-18:1 at a ratio of 1:1 over 9 d had increased urinary concentrations of 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$ (44; J Kraft, unpublished results, 2004). In a study of humans who consumed 11*t*-18:1 (dosage of 1.5, 3.0, and 4.5 g/d over 9 d), Turpeinen et al (21) observed a significant increase in the urinary concentration of 8-iso-$PGF_{2\alpha}$ when they compared the postintervention and adaptation periods ($\bar{x}$: 0.50 and 0.29 nmol/mmol Cr, respectively).

In the current study, the test group's urinary 8-iso-$PGF_{2\alpha}$ concentration after supplementation with 11*t*- and 12*t*-18:1 over 42 d was significantly higher then its baseline concentration. This group's postintervention urinary 8-iso-$PGF_{2\alpha}$ concentration also was significantly higher than that of the control group (Table 2). A more distinctive increase in the urinary 8-iso-$PGF_{2\alpha}$ concentrations was detected in the female subjects than in the male subjects.

In contrast to the current study, Tholstrup et al (45) reported no significant difference in urinary 8-iso-$PGF_{2\alpha}$ concentrations between subjects who consumed a diet containing a naturally 11*t*-18:1–enriched butter (3.6 g 11*t*-18:1/d) supplement and those subjects who consumed a diet low in 11*t*-18:1. Unfortunately, the 12*t*-18:1 content of the butter supplement used in that study was not stated.

Although the *trans* fatty acid intake is associated with inflammatory processes (12) in both the study by Turpeinen et al (21) and the current study, the urinary excretion of 15-ketodihydro-$PGF_{2\alpha}$, which reflects the proinflammatory response (33), was unaffected by supplementation with the *trans* fatty acids. In addition, the biomarkers 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$ correlated with each other after the adaptation period in both treatment groups. After the intervention with the *trans* isomers, the coefficient of correlation between 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$ had decreased significantly in the test group, whereas the correlation coefficient of these biomarkers in the control group was unchanged (Table 3). This trend would suggest that the supplemented *trans* isomers increased the radical-induced lipid peroxidation without influencing the cyclooxygenase-dependent inflammatory response.

Obviously, in studies with 11*t*-18:1 supplementation (21, 44) and in the current study, the portion of 9*c*,11*t*-CLA in serum and RBC membranes was greater (Table 2). Thus, the endogenous conversion of 11*t*-18:1 to 9*c*,11*t*-CLA in the body was proven. However, there was no significant correlation between the greater urinary 8-iso-$PGF_{2\alpha}$ concentration and the greater amount of 9*c*,11*t*-CLA in serum and RBC membranes ($r = -0.245$, $P = 0.468$ and $r = -0.085$, $P = 0.804$, respectively) in any of the studies. Moreover, in the above described studies and in the current study, 11*t*- and 12*t*-18:1 were supplemented together because no highly pure 11*t* 18:1 preparations (ie, those with only a single isomer) in adequate amounts (5 kg) were commercially available.

At present, it is possible that the two *trans* isomers, 11*t*- and 12*t*-18:1, in combination or alone can induce an increase in the

8-iso-$PGF_{2\alpha}$ response, the biomarker of nonenzymatic lipid peroxidation type of oxidative stress. In addition, it is possible that 9*c*,11*t*-CLA, endogenously synthesized from Δ9-desaturation of 11*t*-18:1, was responsible for the observed increase in this biomarker. The induction of lipid peroxidation during CLA supplementation is supported by previous studies in humans (37–39). However, in those studies, the increases in 8-iso-$PGF_{2\alpha}$ (eg, from 0.5 to 1.7 nmol/mmol Cr) and in 15-ketodihydro-$PGF_{2\alpha}$ (eg, from 0.7 to 1.3 nmol/mmol Cr) were more distinct. The comparison of the 9*c*,11*t*-CLA dose supplied via endogenous synthesis in this study (≈20–25% of dietary 11*t*-18:1; 22) with the doses in CLA supplementation studies previously mentioned (isomer mixture containing 10*t*,12*c*-CLA) showed a significantly higher supplementation dose in the CLA supplementation studies (≈2.2–4.2 g/d). Moreover, the prooxidative effect of 10*t*,12*c* CLA is more pronounced than that of 9*c*,11*t* CLA (46–48). Apparently, the higher the 10*t*,12*c* portion of the CLA mixture, the more pronounced the lipid peroxidation.

A meta-analysis of 60 controlled trials in humans found that the ratio of total to HDL cholesterol was higher during consumption of *trans* fatty acids than during consumption of *cis*-unsaturated fatty acids (49). However, in the current study, after consumption of the diet enriched with 6 g 11*t* and 12*t* 18:1 over 42 d, the atherogenic risk ratio compared with that in the control group was unchanged (total:HDL cholesterol, 3.35 ± 0.66 in the test group and 3.21 ± 0.53 in the control group; $P = 0.863$).

In general, concentrations of 8-iso-$PGF_{2\alpha}$ are elevated in conditions thought to be associated with free radical–induced oxidative injury in humans, such as smoking, hypercholesterolemia, diabetes mellitus, overweight, and obesity (3, 32). At present, the clinical relevance of higher 8-iso-$PGF_{2\alpha}$ concentrations in urine and in plasma, in particular after CLA and 11*t*,12*t*-18:1 supplementation in healthy subjects, is unclear. Kumar et al (50) proposed that greater lipid peroxidation could stimulate endogenous defense systems and indicated a potential antiinflammatory effect of 8-iso-$PGF_{2\alpha}$ in the microvasculature. In general, the role of CLA in oxidative stress is controversial (51). Some authors state that CLA has prooxidative properties, which are responsible for the CLA-induced anti-cancer activity (52, 53). In contrast, CLA reduced lipid peroxidation in animal studies and may have antioxidative properties associated with scavenging radicals (43, 54).

Dietary fats can induce oxidative DNA damage in different matrixes. A diet high in fat increased the urinary excretion of 8-oxodG in rats (55), whereas the extent of unsaturation was related to the 8-oxodG concentration in mammary gland DNA in the same animals (56). In contrast, de Kok et al (57) observed no significant increase, after supplementation with linoleic acid (7.5 or 15 g/d over 6 wk), in 8-oxoG in DNA from human peripheral lymphocytes. Under physiologic conditions in humans, the urinary 8-oxodG concentration ranges from 0.5 to 1.7 nmol/mmol Cr (58; A Wagner, unpublished observations, 2004), whereas, in cancer patients, smokers, and obese subjects, increased urinary 8-oxodG has been found (1, 59).

The effect of the supplementation with 11*t*- and 12*t*-18:1 on urinary 8-oxodG concentrations has not been previously reported. In the current study, the values of urinary 8-oxodG were within the physiologic range and showed no differences between the sexes or the treatment groups (Table 2). Park and Floyd (4) postulated that lipid peroxidation products mediate the formation of 8-oxodG. In the current study, the greater 8-iso-$PGF_{2\alpha}$ excretion of the test group was not associated with elevated 8-oxodG excretion (Table 3). This lack of association indicates no oxidative effect on DNA during the intervention with 6 g 11*t*/12*t* 18:1.

In addition, modifications in dietary antioxidants—in particular, α-tocopherol and retinol—can induce changes in the levels of biomarkers of oxidative stress (35, 57, 60). Supplementation with high doses of α-tocopherol in rats decreased the basal urine concentration of both 8-iso-$PGF_{2\alpha}$ and 15-ketodihydro-$PGF_{2\alpha}$ (61). In contrast, the CLA-induced increase in urinary 8-iso-$PGF_{2\alpha}$ with additional supplementation could not reduce moderate concentrations of α-tocopherol in humans (47). Mice fed a *trans* diet developed plasma tocopherol depletion accompanied by a higher concentration of plasma F_2-isoprostanes (36). In the current study, despite an increase in 8-iso-$PGF_{2\alpha}$ excretion, no change was found in the plasma concentrations of α-tocopherol (Table 2). The plasma concentrations of retinol were significantly higher in the test group than in the control group, but, in the case of plasma β-tocopherol, the reverse was true. In addition, the comparison of the total tocopherol equivalents and retinol between the treatment groups found no significant differences throughout the study.

After supplementation with 11*t*- and 12*t*-18:1, the biomarker of the free radical–induced lipid peroxidation increased from the baseline concentrations (diet without these *trans* isomers). However, no major effects could be observed on cyclooxygenase-induced lipid peroxidation, DNA damage, or antioxidant status.

In conclusion, with respect to the high intake of *trans* fatty acids in the US and Canadian populations (>5.0 g/d, especially by PHVOs), our findings indicate that *trans* fatty acids could be relevant to the development of the previously mentioned diseases. Although an increase in urinary 8-iso-$PGF_{2\alpha}$ was observed in this study, and the effects of prolongd high consumption of *trans* fat (>5.0 g/d) could be relevant to the development of disease, the intakes of 11*t*- and 12*t*-18:1 in Europeans tend to be estimated as significantly below those in this study (6.0 g/d), which could minimize the possible risk of detrimental effects on human health. Further research is merited to investigate and more clearly define the effects of individual *trans* fatty acid isomers on oxidative stress and their relation to disease.

KK and JK were responsible for the conception and design of the study; KK was responsible for the conduct of the study; KK and AW were responsible for data acquisition; KK was responsible for data analysis; KK, AQ, SB, and GJ were responsible for data interpretation; KK was responsible for the statistical analysis; KK was responsible for drafting the manuscript; KK, AQ, JK, SB, and GJ were responsible for critical revision of the manuscript; and JK was responsible for obtaining funding. None of the authors had any personal or financial conflict of interest.

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CHAPTER 4

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

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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

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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, etc). 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 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 is currently derived from industrially processed food products containing hydrogenated vegetable oils. In contrast, in the European Union about 40 % is 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 cell; SCD, stearoyl-CoA desaturase; $sPLA_2$, secretory phospholipase A_2.

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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.* 2002; 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,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.* 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 cyclo-oxygenase.

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***,

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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 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).

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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 group's 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 intervention treatments were applied (control group

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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	2·823	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.

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	4·84
$CD4^+CD8^+$	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.

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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; Kew, 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/l per 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.

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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 *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.* 2004). 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 membrane 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 3·1 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.* 2002). Tholstrup *et al.* (2006) reported in a butter supplemen-

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tation 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, 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:HDL-cholesterol ratio and the LDL-cholesterol:HDL-cholesterol ratio were not affected by the study treatments. In the study with 3·1 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 activation, respectively (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.

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CHAPTER 5

Gender-specific gene expression in human monocytes in relation to dietary intervention with two *trans* fatty acids (*t*11 and *t*12 18:1)

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CHAPTER 5

Gender-specific gene expression in human monocytes in relation to dietary intervention with two *trans* fatty acids (*t*11 and *t*12 18:1)

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Running title: *t*11/*t*12 intervention - gene expression

Key words: *trans* fatty acids, conjugated fatty acids, gene expression, peroxisome proliferator-activated receptors, gender

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ABSTRACT

BACKGROUND Gene-by-diet interactions play an important role for the prevention of several diseases such as atherosclerosis in developed societies. CLA (conjugated linoleic acids) are potent ligands of important gene regulators such as peroxisome proliferator-activated receptors (PPAR). According to the dietary supplementation of two *trans* (*t*) isomers (*t*11 and *t*12 18:1), the *c*9,*t*11 CLA significantly increased, most likely due to the Δ9-desaturation of *t*11 *in vivo*.

OBJECTIVE The determination of marker genes in isolated monocytes during the intervention with two *trans* isomers (*t*11/*t*12) and the *c*9,*t*11 CLA synthesis *in vivo*.

DESIGN After the supplementation-free adaptation period (baseline), the test female ($n = 6$) and test male subjects ($n = 6$) received 3.0 g *t*11 and 3.0 g *t*12 18:1/d (Σ 6.0 g/d) for a six wk intervention period. The female and male control subjects ($n = 12$) consumed a control oil. During the entire study all subjects consumed a basal diet and at the end of each study period all subjects received a standardized diet, both low in *t*FA and CLA. The expression of some candidate genes was determined by q-RT-PCR in isolated monocytes of female and male subjects.

RESULTS The gene expression was different between both gender and treatment subgroups. In relation to the *t*11/*t*12 intake the *cyclooxygenase 2, fatty acid translocase,* and *B-cell leukemia 2* were induced in female test subjects compared to the adaptation period ($P \leq 0.001$). In male test subjects the *peroxisome proliferator-activated receptor* γ (*PPARγ*), *glucose transporter 4* (*GLUT4*), and *peroxisomal bifunctional enzyme* were significantly increased compared to baseline and were significantly higher compared to the male control group ($P \leq 0.001$). In male control subjects *PPARγ, GLUT1,* and *GLUT4* were down-regulated. In female control subjects numerous genes were significantly increased in monocytes compared to baseline.

CONCLUSION The results indicate a gender-related gene expression in monocytes during the *t*11/*t*12 supplementation. The effects in test subjects can be attributed to *t*11 and/or *t*12 as well as to the endogenously synthesized *c*9,*t*11 CLA.

INTRODUCTION

High *t*FA intake is suggested to have adverse effects on blood lipids and is related to an increased risk of cardiovascular diseases (Ascherio, 2006; Lemaitre *et al.* 2006). Recent findings demonstrate a route for the *de novo* synthesis of *c*9,*t*11 CLA (conjugated linoleic acid) from *t*11 in human tissues *via* Δ9-desaturation by stearoyl-CoA desaturase (SCD; Turpeinen *et al.* 2002, Kuhnt *et al.* 2006*a*). CLA, generally formed by rumen bacteria from PUFA, have unique metabolic properties, such as anti-cancerogenic, anti-atherogenic, and anti-inflammatory, especially as observed in animal models (Wahle *et al.* 2004).

In recent years, it was shown that nucleus-targeted signaling of fatty acids is attained with the help of fatty acid binding proteins (*FABP*), responsible for transport of fatty acids from cytoplasm to nucleus, and *via* PPAR, transcription factors accepting fatty acids as agonists (Wolfrum *et al.* 2001, Guthmann *et al.* 2004, Adida & Spener, 2006; FIGURE 1). PPAR (subtypes α, β/δ, and γ) target genes are generally involved in lipid and energy metabolism. PPARα (fatty acid oxidation) and PPARγ (storage of fatty acids) target genes are directly implicated in lipogenic pathways such as fatty acid transport protein (*FATP*), *GLUT1*, *GLUT4*, *acyl-CoA oxidase* (*ACOX1*), *malic enzyme* (*ME*), and *lipoprotein lipase* (*LPL*; Kersten, 2000; Wolfrum *et al.* 2001, FIGURE 1).

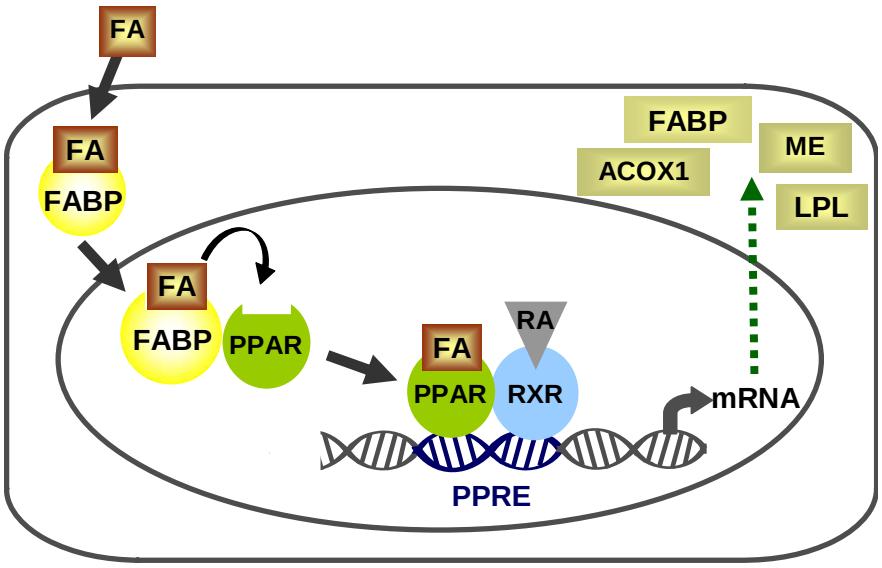

FIGURE 1 Fatty acid (FA) signaling to the nucleus (Wolfrum *et al.* 2001). PPAR ligands bind to fatty acid binding proteins (FABP) to be transported to the nucleus. During gene regulation, PPAR bind to retinoic-acid X receptor (RXR) which in turn need to be activated by *cis*9-retinoic acid (RA) to affect gene transcription. Further, PPAR/RXR heterodimers bind to the peroxisome proliferator responsive element (PPRE) in the promoter region for gene regulation.

CLA as minor dietary fatty acids are naturally occurring ligands and activators of PPAR subtypes (Moya-Camarena *et al.* 1999, Belury *et al.* 2002, Yu *et al.* 2002). The *t*11 was shown to be a ligand of PPAR subtypes as well (Benjamin *et al.* 2005).

During this intervention study, it was shown that subjects of the test group synthesized *c*9,*t*11 CLA *in vivo* with *t*11 as precursor. In respect of cellular signaling mechanisms of CLA and other fatty acids we determined the expression of various genes involved in lipid and glucose metabolism which are partly regulated by PPAR or which have a PPRE in their promoter in isolated monocytes of the subjects.

SUBJECTS AND METHODS

Subjects and study design

24 healthy subjects (12 women and 12 men, mean age 24 ± 3 years) were recruited. The volunteers were informed of the purpose, course, and possible risks of the study. All subjects gave their written consent prior to participation. The study was approved by the ethics committee of the Friedrich Schiller University of Jena. Subjects had no diagnosed diseases, were not taking any medications, e.g., acetylsalicylic acid, were not abusers of alcohol, and took no dietary supplements. The subjects had normal body weight, BMI, and serum lipid profile at baseline (TABLE 1). Gender numbers were balanced in each study group which consisted of six men and six women.

TABLE 1

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

	Women (n = 12)	**Men** (n = 12)	***P* for gender**[†]
Age [y]	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 ± 2.0	22.0 ± 2.0	0.011
Body fat mass [%]*	22.4 ± 4.5	15.8 ± 5.8	0.005
Plasma			
Total C [mmol/L]	178 ± 48	171 ± 65	NS
LDL-C/HDL-C	122 ± 48	159 ± 48	NS
Triacylglycerols [mmol/L]	1.0 ± 0.3	1.0 ± 0.4	NS
Leptin [ng/dL]	8.7 ± 4.9	2.2 ± 1.5	0.001
Adiponectin [mg/dL]	5.9 ± 2.2	7.9 ± 2.1	0.042
Urine			
8-iso-$PGF_{2\alpha}$ [mmol/mmol Cr]	0.45 ± 0.13	0.39 ± 0.16	NS
15-kd-$PGF_{2\alpha}$ [mmol/mmol Cr]	0.21 ± 0.06	0.20 ± 0.09	NS

All values are means ± SD. BMI, body mass index; C, cholesterol; Cr, creatinine; NS, not significant; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; 15-kd-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$. [†] *t*-test, $P \leq 0.05$. * BIA 2000-C (Data Input GmbH, Darmstadt, Germany).

The blind intervention study with parallel design started with a two-wk adaptation period. Both treatment groups (control and test group) received a diet added with pure chocolate spread, free of supplements, for achieving baseline levels (period I). During the following intervention period (period II) the test group diet was supplemented with daily 3.0 g *t*11 and 3.0 g *t*12 (total 6 g/d *t*11/*t*12; Natural ASA, Hovdebygda, Norway) whereas the control group diet was supplemented with a control oil, which was CLA- and *t*FA-free. During the entire study subjects were instructed to consume a diet marginal in *t*FA and CLA. All subjects received a standardized diet (also low in *t*FA and CLA) over the last week of each study period at time of blood sampling. The analytical methods for the determination of food composition and intake are described (Kuhnt *et al*. 2006*a*). All volunteers consumed daily 20 g of a pure commercial chocolate spread (18:1 60%, 16:0 18%, 18:2 13%; % of FAME) to make the adaptation diet isocaloric compared to the intervention diet. The control oil was almost similar to the chocolate spread (18:1 50%, 16:0 14%, 18:2 12%; % of FAME). Both preparations – control oil and *trans* isomers – were added to the chocolate spread to achieve a good acceptability during the intervention period. Each subject consumed daily 20 g of the mixture of chocolate spread and the respective preparations (*trans* isomers or control oil).

Blood sampling

Blood samples (20 mL) were collected on the last day of standardized diet of the adaptation period (day 0, baseline) 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).

Immunophenotyping

The distribution of lymphocytes, monocytes, and granulocytes in blood samples was determined with flow cytometry (two-colour immunophenotyping, flow cytometer FACScan™ and simulSET™ software; BD Biosciences, Heidelberg, Germany).

Preparation of peripheral blood mononuclear cells

Fresh blood was diluted with phosphate buffered saline (PBS, 5 mL), layered carefully onto Histopaque®-1.077 (density 1.077 g/L, Sigma-Aldrich, Munich, Germany) in equal volumes (15 mL), and centrifuged for 30 min at 400 x g at room temperature. The upper layer containing PMNC (a mixture of monocytes and lymphocytes) was aspirated and washed twice

with PBS (centrifugation at 250 x g, 10 min) to lower the degree of erythrocyte contamination.

Isolation of monocytes and quantitative RT-PCR

Monocytes were isolated immuno-magnetically using CD14 micro beads by positive selection method (Miltenyi Biotec, Bergisch Gladbach, Germany). The purified monocytes (above 90-95% purity, per FACS analysis, BD Biosciences) were immediately lysed in the RLT lysis buffer (Qiagen RNeasy kit, Hilden, Germany). Total RNA was isolated according to the manufacturer's protocol (Qiagen). 1-2 µg total RNA was obtained per 1×10^6 cells. Samples were pooled by criteria of gender, test and control groups. On the basis of pooled samples, differential gene expression in monocytes can be compared disregarding individual variability.

Quality (i.e. 18S/28S RNA ratio >1.8) and quantity of pooled RNA samples were controlled using Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) according to manufacturer's instructions. The expression of candidate genes was determined with quantitative real-time reverse-transcription PCR (q-RT-PCR; TABLE 2). One µg total RNA in 20 µL was reversely transcribed to cDNA using 10 mM dNTP mixture (GeneCraft, Münster, Germany), 250 ng random hexamer primers and 200 U Superscript II RNase H^- (both from Invitrogen, Karlsruhe, Germany). Thermal cycler (Biometra, Göttingen, Germany) conditions for reverse transcription were according to Invitrogen's protocol.

For qRT-PCR analysis, target gene primers were designed by Primer Express 2.0 software (Applied Biosystems, Darmstadt, Germany) and synthesized by Proligo (Paris, France). A total volume of 25 µL/well reaction mixture containing diluted cDNA (corresponding to 25 ng total RNA), 200 nM forward and reverse primer mixture (TABLE 2), and 12.5 µL SYBR Green PCR Master Mix was processed for singleplex amplification. PCR was carried out on a 96-well GeneAmp 5700 Sequence Detection System. Cycling parameters were according to manufacturer's standard conditions.

The C_t values obtained from q-RT-PCR measurements represent PCR cycles (C_t) normalized to β-actin as house keeping gene (ΔC_t). In the figures data are presented as 15 minus (ΔC_t) values.

TABLE 2

Forward and reverse primers of candidate genes used in quantitative (q) RT-PCR.

Candidate marker gene		Forward primer[1]	Reverse primer[1]	No.[†]
β-Actin	*housekeeping gene*	5'-CGTCCACCGCAAATGCTT-3'	5'-GTTTTCTGCGCAAGTTAGGTTTTGT-3'	001101
CD14	*monocyte differentiation antigen*	5'-GGGCTTTGCCTAAGATCCAAGA-3'	5'-GTTTTCTGCGCAAGTTAGGTTTTGT-3'	000591
PPARα	*peroxisome proliferator-activated receptor*	5'-ATCCCAGGCTTCGCAAACTT-3'	5'-CATGGCGAATATGGCCTCAT-3'	005036
PPARβ		5'-CGTACGATCCGCATGAAGCT-3'	5'-CTGGCACTTGTTGCGGTTCT-3'	006238
PPARγ		5'-CCAAGGCTTCATGACAAGGG-3'	5'-GCAAACTCAAACTTGGGCTCC-3'	138711
RXR	*cis-9 retinoic acid-X-receptor*	5'-GGGCTGGGACTGTTTCGTTT-3'	5'-CATCGTCTGTCCTGGCGTTT-3'	002957
A-FABP	*adipocyte-type fatty acid binding protein*	5'-GGGCCAGGAATTTGACGAAG-3'	5'-TGTACCAGGACACCCCCATC-3'	001442
E-FABP	*epidermal-type fatty acid binding protein*	5'-TTTGAAGAAACCACAGCTGAT-3'	5'-CTCCTGATGCTGAACCAATGC-3'	001444
FAT (CD36)	*fatty acid translocase*	5'-GGAAAATGTAACCCAGGACGC-3'	5'-GATAGTGAAGGTTCGAAGATGGCA-3	000072
FATP	*fatty acid translocase protein*	5'- GCCGGACGTTTGACCAGAT-3'	5'-GTGCTGGGTTTCACCTCCTG-3'	005094
GLUT1	*glucose transporter1*	5'-GAGTTGGCGCTGTAAACATGG-3'	5'-GCACAAGTCCCACTGACATGAA-3'	000340
GLUT4	*glucose transporter4*	5'-GCCGGACGTTTGACCAGAT-3'	5'-GTGCTGGGTTTCACCTCCTG-3'	001042
SCD	*stearoyl-CoA-desaturase*	5'-ACCGCTGGCACATCAACTTC-3'	5'-CCTTGGAGACTTTCTTCCGGTC-3'	005063
COX2	*cyclooxygenase2*	5'-CCAAGCTTTCCTGCTCAGTGTT-3'	5'-CCCCCAGTCCCTTTTCTTCA-3'	000963
CYP4A11	*cytochrome p450*	5'-CACCACAGCCAGTGGGATC-3'	5'-CCGGCACCTCTCCTGATG-3'	000778
LPL	*lipoprotein lipase*	5'-GTGAAATGCCATGACAAGTC-3'	5'-CACATGCCGTTCTTTGTTC-3'	000237
ACOX1	*acyl-CoA oxidase1*	5'-CCAAGCTTTCCTGCTCAGTGTT-3'	5'-CCCCCAGTCCCTTTTCTTCA-3'	007292
PBE	*peroxisomal bifunctional enzyme*	5'-GGGTAGGATTCACAAACC-3'	5'-GGTACGTGGTTCAATGTG-3	001966
ME	*malic enzyme (malate dehydrogenase)*	5'-GATCCGGCACATCCCAG-3'	5'-GCTGCTCAGAGACTTCC-3'	006680
BCL2	*B-cell CLL/lymphoma 2*	5'-GCTGGCTCAGGACTATCTGCA-3'	5'-TGTAGCACTCTGGACGTTTTGC-3'	000633
PCNA	*proliferating cell nuclear antigen*	5'-CCTGGTCCAGGGCTCCA-3'	5'-CAGCAGGCCTCGTTGATG-3'	002592

[1] Designed by Primer Express 2.0 software (Applied Biosystems), [†]Gene Bank accession No.: NM_ .

Statistics

All statistical analyses were performed using the SPSS software version 14.0 (SPSS Inc, Chicago, USA). Differences with a P value ≤ 0.05 were regarded as significant. The data values are stated as means with their standard deviations (SD). The dietary food intake, immune cell distribution, and subject´s baseline data were analyzed with treatments and gender as factors using t-test. The results of pooled samples (n = 5 independent analyses) within one group (intra-group) were compared using t-test with treatments and genders as factors as well. The analysis of covariance (ANCOVA) was used to compare data of gene

expression of the two treatments within one gender after intervention period (inter-group). The baseline value was used as covariate. Differences of data regarding gene expression with a P value ≤ 0.001 were regarded as significant.

RESULTS

Diets

As a result of the intake of the preparation mixtures during the intervention period the fat intake increased in both treatment groups to a similar extent (TABLE 3).

TABLE 3
Daily dietary intake during the standardized diet of the study.

Daily intake		Control group		Test group	
		Adaptation period	Intervention period	Adaptation period	Intervention period
Energy [MJ]	**w**	8.6 ± 1.9	8.7 ± 1.1	9.3 ± 1.5	8.5 ± 2.3
	m	11.3 ± 1.1*	11.4 ± 0.5*	10.6 ± 1.4*	10.9 ± 1.3*
Carbohydrates [g/d]	**w**	319 ± 47	306 ± 47	341 ± 65	289 ± 100
	m	393 ± 47*	389 ± 25*	390 ± 56*	371 ± 64*
Carbohydrates [en%]	**w**	62.9 ± 4.1	60.1 ± 3.4	63.2 ± 3.1	59.5 ± 4.0
	m	59.8 ± 1.8	58.7 ± 1.2	62.9 ± 3.8	60.4 ± 4.0
Protein [g/d]	**w**	57 ± 8	58 ± 8	64 ± 9	58 ± 11
	m	80 ± 7*	80 ± 3*	74 ± 12*	73 ± 11*
Protein [en%]	**w**	12.0 ± 1.2	11.9 ± 1.1	12.3 ± 0.6	12.2 ± 1.1
	m	12.7 ± 0.2	12.4 ± 0.3	12.3 ± 0.5	12.3 ± 0.5
Fat [g/d]	**w**	58 ± 10^{b}	64 ± 12^{a}	62 ± 11^{b}	66 ± 13^{a}
	m	79 ± 5^{a*}	81 ± 8^{b*}	76 ± 15^{a*}	83 ± 5^{b*}
Fat [en%]	**w**	26.9 ± 3.2^{b}	29.5 ± 2.9^{a}	26.1 ± 2.7^{b}	30.0 ± 3.3^{a}
	m	29.0 ± 1.5^{b*}	30.6 ± 0.8^{a}	27.4 ± 4.1^{b}	29.1 ± 4.5^{a}
		Fatty acids [% of fat intake]			
Total SFA	**w**	25.4 ± 2.5	26.3 ± 2.0	26.4 ± 1.8	25.5 ± 2.7
	m	24.8 ± 3.2*	26.0 ± 3.0	25.8 ± 3.0	24.9 ± 2.8
Total *cis* MUFA	**w**	34.1 ± 1.7	33.9 ± 1.7	33.1 ± 2.5^{a}	31.4 ± 2.2^{b}
	m	31.9 ± 0.3*	32.1 ± 0.9*	31.8 ± 3.2	30.9 ± 2.4
Total *trans* MUFA	**w**	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{b}	9.5 ± 1.8^{a}
	m	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	7.3 ± 1.1^{a}
Total PUFA	**w**	26.5 ± 3.6^{a}	26.9 ± 3.5^{a}	26.3 ± 3.0^{a}	22.1 ± 2.7^{b}
	m	30.0 ± 2.6^{a*}	30.4 ± 4.9^{a*}	29.0 ± 1.8^{a*}	25.2 ± 5.5^{b*}
18:0	**w**	5.6 ± 0.5	5.6 ± 0.6	5.6 ± 0.5	6.3 ± 0.7
	m	6.3 ± 0.5	6.2 ± 0.1	5.9 ± 0.6	6.5 ± 0.7
***c*9 18:1**	**w**	30.2 ± 1.4^{a}	29.2 ± 1.6^{ab}	29.1 ± 2.6^{b}	24.4 ± 1.9^{c}
	m	27.0 ± 0.3*	27.3 ± 0.8*	28.7 ± 2.5	24.1 ± 1.8
***c*9,*c*12 18:2**	**w**	23.2 ± 3.7^{a}	23.5 ± 2.9^{a}	23.4 ± 3.0^{a}	19.5 ± 3.1^{b}
	m	27.7 ± 2.6^{a*}	27.8 ± 1.8^{a*}	26.8 ± 5.4^{a*}	22.4 ± 6.0^{b*}
***c*9,*c*12,*c*15 18:3**	**w**	1.9 ± 0.2	2.1 ± 0.1	2.1 ± 0.3	1.8 ± 0.2
	m	1.9 ±0.2	2.0 ± 0.2	1.8 ± 0.1	1.7 ± 0.2
TE [mg/g fat]	**w**	0.20 ± 0.04	0.18 ± 0.04	0.23 ± 0.04	0.21 ± 0.04
	m	0.23 ± 0.02	0.21 ± 0.01	0.21 ± 0.03	0.20 ± 0.02

All values are means ± SD. w, women; m, men; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; en %, percentage of energy intake; TE, tocopherol equivalents. ^{abc} Mean values within a row with different superscript letters were significantly different (t-test, $P \leq 0.05$). * Mean values between gender-related subgroups were significantly different (t-test, $P \leq 0.05$). 1 mg TE = 1 mg α-tocopherol = 10 mg γ-tocopherol = 1.49 IU.

The portions of carbohydrates, protein, and fat of total energy were not different between both treatment groups and gender. The *t*FA and CLA intake was low as planned during the standardized diet. Due to the intake of the *t*11/*t*12 mixture in the test group the portion of *trans* MUFA was increased. This resulted in a significantly lower intake of *cis* MUFA and total PUFA compared to baseline and control group. Male subjects had generally higher daily food intake than women which was independent on treatment group (TABLE 3).

Immune cell distribution

In male subjects, no differences between control and test group were observed during adaptation and intervention periods with respect to lymphocyte and monocyte percentages (TABLE 4). In contrast, the percentage of granulocytes significantly increased in the male test group after the intervention period. Moreover, in comparison to the male, among the female groups only the percentage of monocytes differed and was in the control group significantly lower after the intervention period than after the adaptation period, while for the test group no changes occurred. These intra- and inter-gender differences indicate period- and gender-related effects on blood immune cell populations (TABLE 4).

TABLE 4

The distribution of lymphocytes, monocytes, and granulocytes in blood samples of male and female subjects during the study.

Plasma		**Control group**		**Test group**	
		Adaptation period	Intervention period	Adaptation period	Intervention period
Lymphocytes [%]	w	37.5 ± 7.4	38.8 ± 5.9	43.2 ± 12.1	43.3 ± 12.0
	m	39.8 ± 10.5	39.7 ± 8.6	40.8 ± 8.0	34.8 ± 6.0
Monocytes [%]	w	5.9 ± 1.1^{a}	4.8 ± 1.3^{b}	5.2 ± 0.9^{b}	5.5 ± 0.6^{ab}
	m	6.8 ± 0.9*	6.7 ± 0.8*	6.8 ± 1.3*	6.8 ± 0.8*
Granulocytes [%]	w	56.7 ± 8.1	57.0 ± 5.3	51.5 ± 12.7	51.3 ± 11.8
	m	53.3 ± 9.9^{ab}	53.8 ± 9.1^{ab}	51.8 ± 7.9^{b}	58.5 ± 5.3^{a}

All values are means ± SD. w, women; m, men. ^{abc} Mean values within a row with different superscript letters were significantly different (*t*-test; $P \leq 0.05$). ***** Mean values between gender-related subgroups were significantly different (*t*-test, $P \leq 0.05$).

Gene expression

Twenty-one candidate genes, all involved in lipid and glucose metabolism were chosen on the basis of proteins involved in fatty acid signaling, whose genes are partly regulated by PPAR and have a PPRE in their promoter.

In general, gender-related differences in gene expression were shown. After the intervention period with baseline as covariate, in the test group, *GLUT4, E-FABP,* and *A-FABP* were significantly different between male and female test subjects at the end of intervention period (data not shown, baseline as covariate, $P \leq 0.001$).

In the control group, most of the genes were gender-related different at the end of the intervention period, except *FAT, FATP4, PPARβ, COX2, LPL, PCNA,* and *ME* (data not shown, baseline as covariate, $P \leq 0.001$). Therefore, data were shown separated by gender.

Female subgroups

After the adaptation period, gene expression of *PPARγ, GLUT4, FAT,* and *BCL2* was differentially regulated among the later female subgroups (data not shown).

In the female test group, the 15-(ΔC_t) values of *FAT*, *COX2,* and *BCL2* were significantly increased. These genes were significantly induced in test women after the *t*11/*t*12 intervention period (intra-group, $P \leq 0.001$; FIGURE 2).

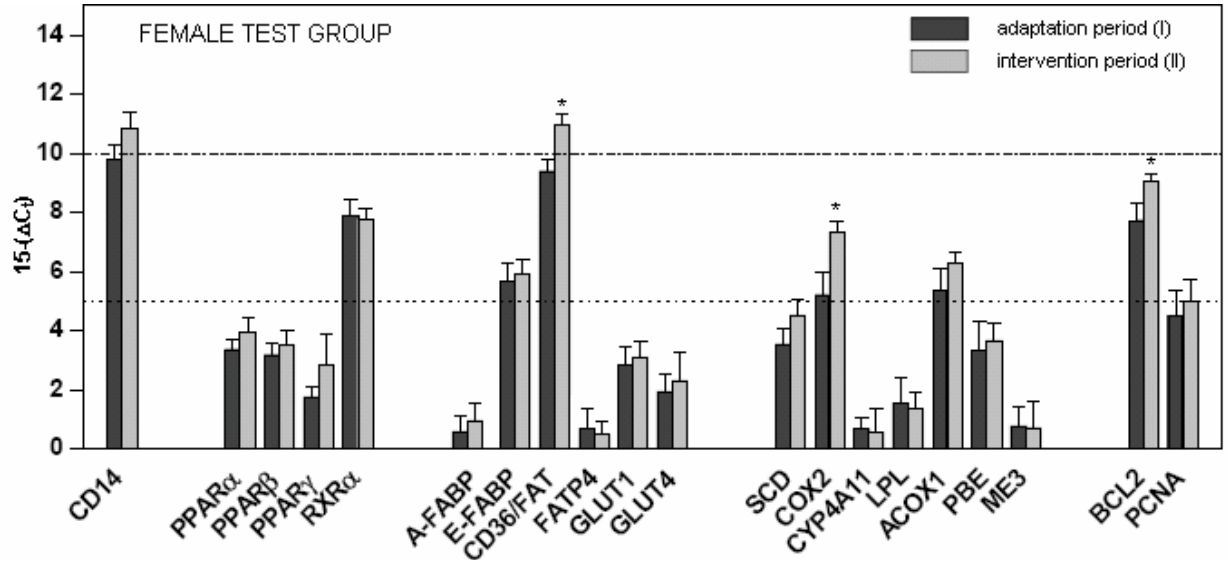

FIGURE 2 Comparison of gene expression of female test subjects after intervention period (II) compared to adaptation period (I). Mean ± SD; $^{*}t$-test ($P \leq 0.001$).

In the female control group an unexpected high gene expression of numerous genes has been determined (intra-group, $P \leq 0.001$; FIGURE 3).

The induction of *PPARγ* and *GLUT4* was exceedingly high. In addition, *PPARα* and *β, E-FAPB, GLUT1, SCD, CYP4A11, ACOX1*, and *PBE* were induced despite the *t*FA- and CLA-free control diet (FIGURE 3).

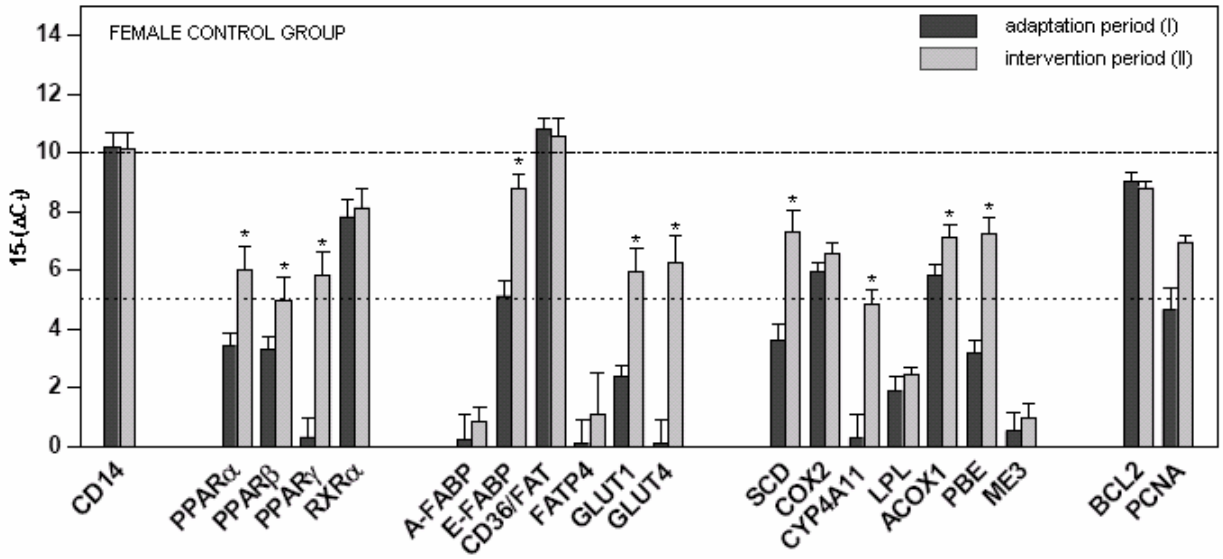

FIGURE 3 Expression of candidate genes of female control subjects after intervention period (II) in relation to the adaptation period (I). Mean ± SD; * *t*-test ($P \leq 0.001$).

The comparison of gene expression of both groups after intervention period was analyzed using the gene expression at baseline as covariate. Due to the high gene induction in female control group most of the genes are higher in the female control group compared to the female test group. In exception, *BCL2* ($P = 0.009$), *FAT* ($P = 0.024$), and *COX2* ($P = 0.048$), which were increased in test females, were higher compared to the female control group (inter-group, FIGURE 4).

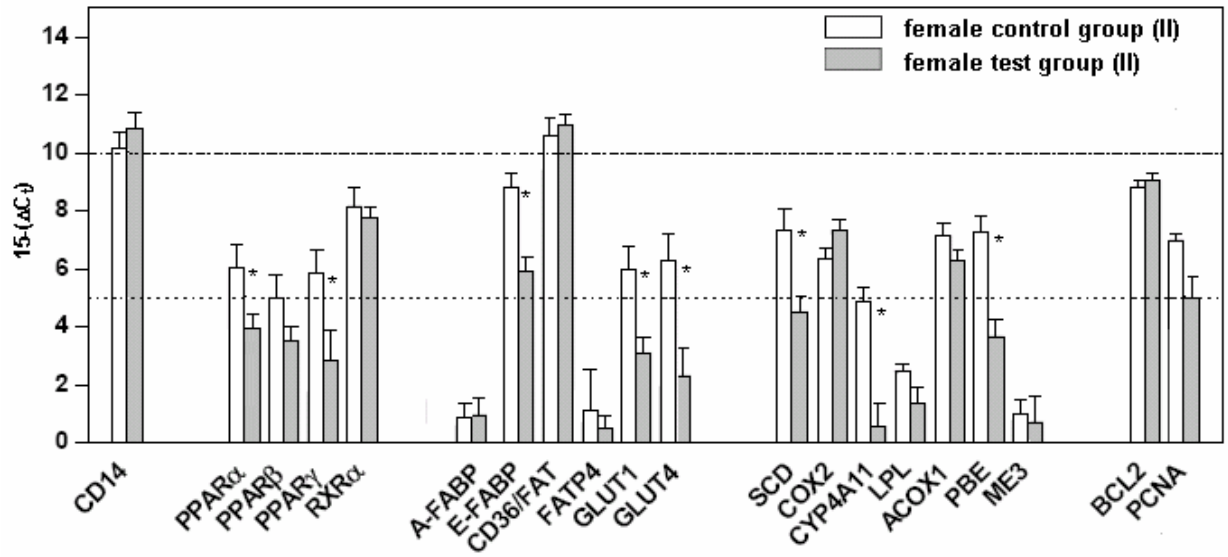

FIGURE 4 Comparison of gene expression of female control and test subjects after intervention period (II) with baseline as covariate. Mean ± SD; *ANCOVA ($P \leq 0.001$).

Male subgroups

In general, the gene expression of *PPARγ, GLUT4,* and *FAT* as in women and of further genes such as *PBE* and *E-FABP* were differentially regulated among male subgroups at baseline after adaptation period, although all male subjects consumed similar diets as well.

Due to the consumption of both *trans* isomers, in male test subjects *PPARγ*, *GLUT4,* and *PBE* were significantly increased ($P \leq 0.001$; FIGURE 5).

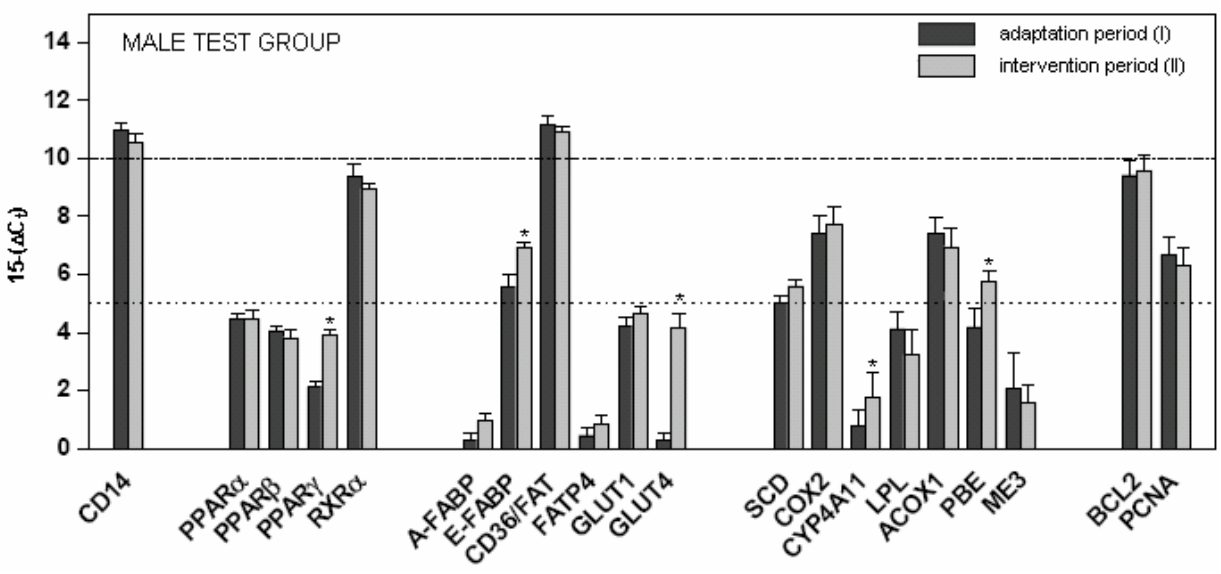

FIGURE 5 Comparison of gene expression of male test subjects after intervention period (II) compared to adaptation period (I). Mean ± SD; $^{*}t$-test ($P \leq 0.001$).

Contrary to female control subjects, in male controls only a minimal gene regulation related to the *t*11/*t*12- and *c*9,*t*11 CLA-free control diet –as expected– was observed.

Compared to baseline *PPARγ, GLUT1, GLUT4*, *FAT*, and *E-FABP* were significantly decreased. In contrast, *BCL2* was increased compared to the adaptation period ($P \leq 0.001$; FIGURE 6).

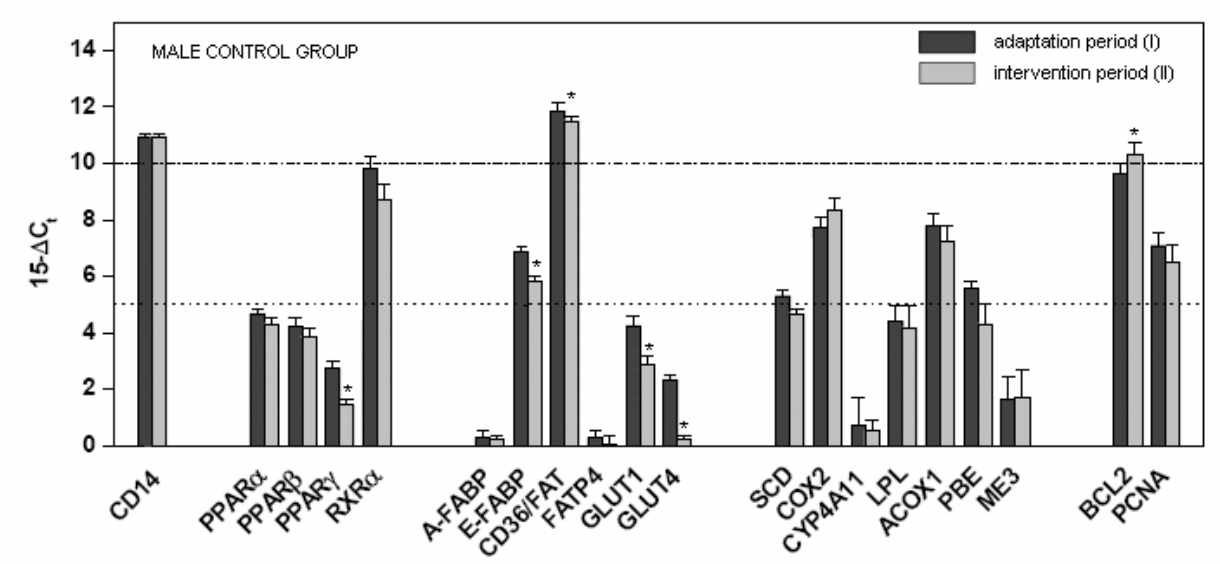

FIGURE 6 Comparison of gene expression of male control subjects after intervention period (II) compared to adaptation period (I). Mean ± SD; $^{*}t$-test ($P \leq 0.001$).

Compared to male test subjects, *PPARγ, GLUT1, GLUT4, A-FABP, SCD,* and *PBE* were significantly lower in the male control subjects after intervention period (FIGURE 7). The *PPARγ*, *GLUT4*, and *PBE* expression was increased in test males and contrary in control males whose expression was decreased. Thus, these genes can be considered as marker genes of *t*11 and *t*12 intake in male subjects.

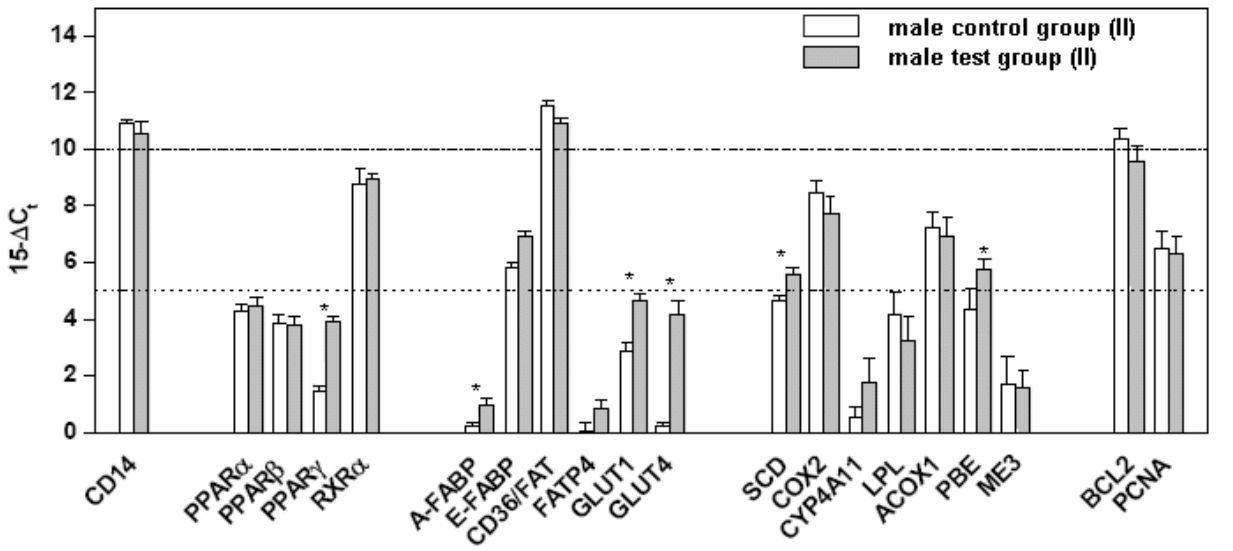

FIGURE 7 Comparison of gene expression of male control and test subjects after intervention period (II) with baseline as covariate. Mean ± SD; *ANCOVA ($P \leq 0.001$).

DISCUSSION

During the intervention study, the *t*11 was evaluated as a potent precursor for *c*9,*t*11 CLA synthesis *in vivo*. In contrast, the *t*12 isomer was not converted to *c*9,*t*12 18:2 by Δ9-desaturation in the human body (Kuhnt *et al.* 2006*a*). The choice of monocytes was straight-forward, as firstly taking blood samples from humans is minimal invasive, secondly these blood cells are well established targets for gene expression studies (Nagata *et al.* 2003). Moreover, the role of circulating monocytes in lipid metabolism is well documented (Li *et al.* 2004). In the present study, human blood cells (erythrocytes and mononuclear cells) were evaluated as adequate biomarkers for the supplemented fatty acids. The *t*11 and *t*12 as well as the endogenously synthesized *c*9,*t*11 CLA were significantly increased in blood cells (Kuhnt *et al.* 2006*a*,*c*).

The present results of gene expression analyses showed clearly gender-related gene regulation due to dietary fatty acids of both treatment groups (test female, test male, control female, and control male subjects). In female test subjects *COX2, FAT,* and *BCL2* expression was induced while in male test subjects expression of *PPARγ*, *GLUT4*, and *PBE* increased in relation to the dietary *t*11/*t*12 intake. Various genes among gender were regulated in the opposite way.

Gender-related differences, especially concerning lipid metabolism were described earlier and the impact of gender-related metabolic differences apparently increased (Blaak *et al.* 2001, Williams, 2004; Mittendorfer, 2005). Various factors must be considered when evaluating the differences in metabolism between genders. Gender variations in adipose tissue lipolysis, in lipid and glucose metabolism during fasting, and circulating blood lipid concentrations were noted in humans (Mauriège *et al.* 1999, Mittendorfer *et al.* 2001; Williams, 2004). In hamsters, dietary fatty acid-dependent modulation of TAG and cholesterol metabolism has been shown to be gender-related (Morise *et al.* 2006). Differences in phenotype (e.g., body composition, regional fat distribution, aerobic fitness, all of which affect substrate metabolism and their amount) and the endocrine milieu (hormones, their concentration and secretion pattern) are considered causes for sexual dimorphism in metabolism (Mittendorfer, 2005).

Although the mechanisms are mostly unknown, there is evidence for gender-specific post-translational differences like enzyme activities, cellular signal transduction elements, and consequently substrate kinetics (Mittendorfer, 2005).

The present results have shown that gene expression was not only affected by fatty acid treatment, but also occurred at baseline. Although all subjects received a standardized diet with equal dosages of pure chocolate spread without supplement for one wk before analysis expression of *PPARγ, GLUT4,* and *FAT* were different among women and men.

Dietary factors Dietary factors are involved in gene regulation. For minimizing effects of the diet at the time of blood collection the study subjects received a standardized diet for ad libitum consumption. This could result in inter-individual differences as well. However, the mean intake of macronutrients of total energy intake did not differ between both treatment groups and study periods, except the fat intake (TABLE 2). The fat intake increased approximately by 6.0 g related to the corresponding treatment, except for the male control subjects (~2.0 g). If the fat amount of the control oil chocolate-spread mix is not considered, the fat intake was significantly decreased in control men to baseline. In general, women had significantly lower intake of macronutrients during both periods of standardized diet than their male counterparts. However, when related to total energy intake differences did not persist, except for the fat intake of control male subjects during adaptation period (TABLE 2).

Obviously, the female control subjects showed an exceedingly high gene response when compared to baseline (FIGURE 3). In contrast, only *COX2* and *BCL2* were induced in male control subjects (FIGURE 6). The control oil was mixed into pure chocolate spread only to balance energy and fat intake during the intervention period compared to baseline and test group diet. The control oil contained mainly *c*9 18:1 whose intake was increased during the control diet but the total fat intake was increased simultaneously. Thus, the consumed *c*9 18:1 portions of fat intake remained unchanged in both female and male control subjects (TABLE 3). In addition, the male control subjects showed no such high gene induction related to the control diet. This indicated a more pronounced gender-related.

Women´s *cis* MUFA intake (mainly *c*9 18:1) was higher whereas the PUFA intake (mainly *c*9,*c*12 18:2) was lower compared to men of both groups (TABLE 3). In principle, the marginal diet-related differences can only partly originate observed gender-specific gene response. Especially the strong gene expression observed in female control subjects by dietary factors is unlikely.

The percentage of monocytes was significantly decreased after intervention period (TABLE 5). This could additionally support the conspicuous changes in female control subjects compared the other study subgroups.

Further gender-related differences during this study Further gender-related differences were determined in the present study. Female test subjects showed a greater *c*9,*t*11 CLA synthesis from *t*11 than male test subjects (Kuhnt *et al.* 2006*a*). The enzyme responsible for the insertion of a *cis*-double bond in the Δ9 position into *t*11 is the stearoyl-CoA-desaturase (SCD). In humans two isoforms have been characterized (*hSCD1* and *hSCD5*, Wang *et al.*

2005). *hSCD1* has a 85% homology to all rodents *SCD* (Ntambi *et al.* 2004). In the present study, the *hSCD1* isoform (called *SCD*) was determined in study subjects.

Hormonal (Cohen & Friedman, 2004) and dietary regulation (cholesterol, PUFA, tocopherols, and carbohydrates; Ntambi 2004) of the *SCD* isoforms are known. PUFA affected *SCD2* expression in lymphocytes (Sessler *et al.* 1996), therefore in the present study standardized diet was given. CLA mixture (*t*10,*c*12/*c*9,*t*11) showed to suppress the SCD enzyme activity in porcine subcutaneous adipose tissue (Smith *et al.* 2002). But in test subjects the synthesized amount of *c*9,*t*11 CLA is most likely inadequate to suppress the *SCD* and *t*10,*c*12 CLA was completely absent in the present study.

Less is known about gender differences in *SCD* expression. Experiments in mice showed consistently higher basal *SCD1* expression in the liver of female mice than male mice (Lee *et al.* 1996). Despite higher *t*11 conversion rate in women, their *SCD* expression was not significantly induced (FIGURE 2) and was not higher compared to male test subjects.

In contrast, *SCD* was significantly induced in control women. The fat accumulation is associated with higher *SCD* expression (Legrand & Hermier, 1992) and SCD activity (Jones *et al.* 1996). Women of the present study had more body fat than men (TABLE 1), but no higher *SCD* expression than male subjects. The body fat mass between females subgroups did not differ significantly (22.0 ± 5.3% *vs* 22.9 ± 4.0%).

Stored TAG are also associated with the production of hormones such as leptin. Female subjects possessed significantly higher plasma leptin and lower adiponectin concentrations than their male counterparts (TABLE 1). Previous studies indicate an association between leptin and *SCD1* expression which could be a link to hormonal-related gene regulation (Cohen & Friedman, 2004).

Furthermore, female test subjects showed a higher increase of urinary excretion of the isoprostane 8-iso-$PGF_{2\alpha}$ (produced by free radical non-enzymatic lipid peroxidation; Kuhnt *et al.* 2006*b*; women Δ 0.21, men Δ 0.13; mmol/mmol creatinine) than male test subjects. The *COX2* is the inducible isoform and catalyzes the biosynthesis of prostaglandins under acute inflammatory conditions. During this study, in female test subjects the *COX2* was significantly increased due to the *trans*-isomer intake (FIGURE 2). The observed induction of *COX2* and the increased 8-iso-$PGF_{2\alpha}$ excretion, especially in females with an unaffected 15-kd-$PGF_{2\alpha}$ excretion support the earlier hypothesis that endogenous synthesis of 8-iso-$PGF_{2\alpha}$ may activate COX2 (Basu, 2003). In conclusion, in female subjects higher lipid peroxidation levels are supposed. Tocopherol intake and anti-oxidative status in plasma were similar

(Kuhnt *et al.* 2006*b*). Several studies confirm gender-related oxidative status and lipid peroxidation in healthy subjects (Veglia, 2006).

Mittendorfer (2005) supposed that the endocrine milieu is involved in sexual dimorphic lipid metabolism as well. Sexual steroid hormones influence the lipid metabolism in a gender-specific manner. In the present study, all female subjects took oral contraceptives and they are considered to have a ´normal' menstrual cycle (28 d). Different cycle phases have specified hormone levels (Zazulak *et al.* 2006). In relation to the six-wk intervention period, female subjects were actually not at the same cycle phase at the time of both blood collections. This could support considerably the impact of hormone status on gene expression during this study, especially in female control subjects. The concentrations of hormones such as estrogens, testosterone, and insulin were unfortunately not determined during this study. In addition, the number and interaction with estrogen-receptors are important as well.

The inter-individual variance may be partially determined by genetic variants of genes such as polymorphisms encoding metabolic relevant proteins and receptors. Gene polymorphisms are known to be able to influence metabolic response to dietary intervention (e.g., *PPARα*; Paradis *et al.* 2005). A polymorphism of *FABP2*, an intestinal fatty acid binding protein, has been associated with an increased postprandial glucose excursion in response to a *trans* 18:1 test meal compared to homozygous individuals. Thus, any effects of *t*FA may be more pronounced in individuals carrying the changed allele (Lefevre *et al.* 2005).

PPARγ appears to be primarily involved in adipogenesis. Leptin production is under negative control by PPARγ in adipose tissue (Kersten *et al.* 2000) which could in turn explain the gender-related gene expression observed during this study. At baseline, expression of *PPARα, β, γ,* and *GLUT1* in female subjects was lower compared to male subjects ($P \leq 0.001$). *PPARγ* is capable to activate the gene expression of the insulin-dependent glucose transporter *GLUT4* (Wu *et al.* 1998). In the present study, in male test subjects *PPARγ* and *GLUT4* were induced compared to baseline and were significantly higher compared to male control group (FIGURE 5). In female test subjects it was increased as well ($P \leq 0.038$). Contrary, the control diet low in *t*FA and CLA might cause lower *PPARγ* and *GLUT1* expression in male control subjects (FIGURE 6). This indicates an influence on *PPARγ* and its regulated genes by dietary *t*11/*t*12 intake and/or the *c*9,*t*11 CLA synthesis *in vivo,* especially in male subjects.

Monocytes and macrophages play an important role in the immune defense and inflammation by cytokine production. Activation of *PPARγ* in monocytes/macrophages is proposed to reduce cytokine production (Kersten, 2000). The *PPARα* and *β* related inhibition of

macrophage foam-cell formation and atherosclerosis was shown in mice (Li *et al.* 2004). Therefore, as one possible mechanism CLA may act anti-atherosclerotic due to PPAR related suppression of pro-inflammatory cytokines, such as TNFα, IL 6 and IL 8 (Jaudszus *et al.* 2006, Ringseis *et al.* 2006, Kritchevsky *et al.* 2004). Endogenously synthesized *c*9,*t*11 CLA from *t*11 as precursor could regulate a subset of genes involved in inflammation through PPAR activation. In contrast, high *t*FA intake can modulate monocyte and macrophage activity as manifested by increasing the production of cytokines (Han *et al.* 2002) and therefore, is associated with inflammation in humans (Lopez-Garcia *et al.* 2005).

FAT (CD36) is a multifunctional protein involved in uptake of apoptotic cells, transport of fatty acids, angiogenesis, modulation of inflammation, atherosclerosis, diabetes, and cardiomyopathy (Silverstein *et al.* 2000). As a result of the increased intake of *t*11 and *t*12 *FAT* expression in monocytes of test female subjects was significantly increased.

However, in this study, due to the intake of daily 6 g *t*11/*t*12 no changes of biomarkers of inflammation and immune system (e.g., serum lipids and lipoproteins, interleukines, TNFα, phagocytosis) have been observed (Kuhnt *et al.* 2006*c*, under review). It is possible that the increased *c*9,*t*11 CLA could compensate the effects of the supplemented *t*11/*t*12 – if they have any effects – whereby the synthesized *c*9,*t*11 CLA amount *in vivo* (~0.7 g/d) is lower than in previous supplementation studies (2.4 - 3.0 g/d, Tricon *et al.* 2004, Risérus *et al.* 2004).

Dietary fatty acids might be partly involved in gender-specific gene regulation. Further factors such as different hormone levels (sexual and growth hormones), body composition, exercise, fatty acid metabolism can cause the observed gender-related differences of gene expression during this intervention study.

In conclusion, the results reflect gender-related gene regulation. Due to the dietary *t*11/*t*12 intake and endogenously *c*9,*t*11 CLA synthesis *COX2, FAT,* and *BCL2* were significantly increased in female test. *PPARγ* and *GLUT4* were significantly increased in male subjects and were significantly higher compared to male control subjects. The control females showed a high gene expression compared to baseline and female test subjects for unknown reason, most likely partly caused by variations in the endocrine milieu. Hypothetically, the observed effects in test subjects can be attributed to *t*11 and/or *t*12 as well as to the endogenously synthesized *c*9,*t*11 CLA.

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CHAPTER 6

FINAL DISCUSSION

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The previous data support a relationship between the high *t*FA intake and risk of cardiovascular disease but most like with differences among positional isomers of *trans*-double bonds. In addition, the *trans*-isomer distribution in foods differs depending on their source and processing. Foods containing industrially hydrogenated fats and oils have partly high *t*FA content with *t*9 and *t*10 as major *trans* 18:1 isomers. In contrast, ruminant-derived fats contain partly less of *t*FA than industrially hydrogenated fats and oils and contain mainly the *t*11 as major *trans* isomer. The *t*11 is known as the major source of *c*9,*t*11 CLA in milk fat due to their endogenous conversion to *c*9,*t*11 CLA Δ9-desaturation. Therefore, the endogenous *t*11 conversion in humans could be relevant for the CLA supply to the human body.

The Δ9-desaturation of *t*11 and *t*12 to their Δ9-desaturation products *c*9,*t*11 CLA and *c*9,*t*12 18:2 during their simultaneous intake is unknown in humans. Furthermore, the incorporation of both supplemented substrates and their possible desaturation products into human tissue lipids and their potential effects on human health are currently uncertain.

For clarifying these questions two human intervention studies (pre-study and main study) were conducted.

In the pre-study 12 female subjects participated. The pre-study started with a 14-d adaptation period free of supplementation. Data obtained from samples collected during the adaptation period were used as baseline values. The intervention period of the pre-study was altogether 28 d. The female test subjects (n = 6) received a daily dose of 1.2 g *t*11 and of 1.2 g *t*12 according to a usual dietary *t*11 intake in European population (Voorrpis *et al.* 2002). The control group (6 female subjects) received a control oil (rapeseed oil and palm kernel oil; 1:1) to balance the fat intake. During the entire study (6 wks) the basal diet had to be low of *t*FA and CLA from industrial and ruminant fats. In the pre-study, for preliminary investigation of the conversion of *t*11 and *t*12 to *c*9,*t*11 CLA and *c*9,*t*12 18:2 fatty acids were analyzed in serum lipids of the females, respectively (Appendix TABLE A-1). All used foods, test mixtures, and analytical methods were identical to the used foods and methods in the main study (Chapter 2, Kuhnt *et al*. 2006*a*).

During the main study both gender were balanced in each study group (male and female subjects, each group n = 6). Subjects received an almost three times higher dosage of *trans*

isomers ∑ 6.0 g/d (3.0 g *t*11 and 3.0 g of *t*12) over a period of 42 d. The control group received the same control oil as in the pre-study. The baseline data were obtained after a supplementation free adaptation period as well (14 d). In the main study, lipids from serum as well as membrane lipids of RBC and PBMC were analyzed. During the main study the basal diet in both periods had only marginal amounts of CLA and any *t*FA as well (industrial and ruminant derived; Chapter 2, Kuhnt *et al.* 2000*a*).

Conversion of t11 and t12 to their Δ9-desaturation products (c9,t[n] 18:2)

In both studies, in all analyzed test subject lipids (serum, membranes of RBC and PBMC) both *t*11 and *t*12 were increased due to the *trans*-isomer supplementation (FIGURE 7). Therefore test subject´s compliance can be evaluated quite well. In addition, the *c*9,*t*11 CLA content in lipids of serum (FIGURE 7) and membranes of RBC and PBMC was significantly increased in the test subjects while *c*9,*t*12 18:2 was not higher than its initial concentration (Chapter 2, Kuhnt *et al.* 2006*a*). The basal diets were as far as possible free of *t*FA and CLA during both studies, therefore, increased *t*FA and CLA in lipids could mainly have derived from the supplemented *trans*-isomer mixture and the endogenous conversion.

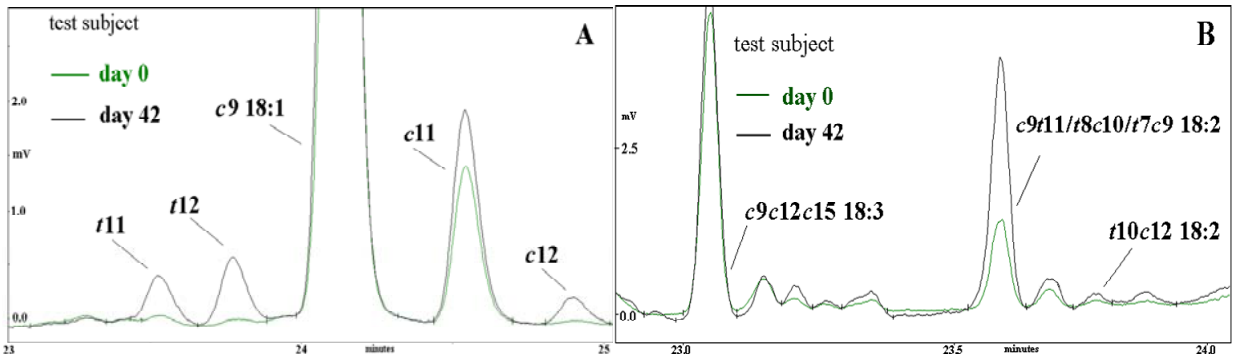

FIGURE 7 Increase of supplemented 18:1 isomers [A] and CLA [B] in serum lipids of a test group subject after adaptation (day 0) and intervention period (day 42). [A] *trans* and *cis* fatty acids of 18:1, capillary column CP-select, length 200 m. [B] section of fatty acid profile; capillary column DB-225ms, 60 m.

The estimated conversion rates of *t*11 and *t*12 were calculated as followes: the net change desaturase product/ (net change desaturase product + net change desaturase substrate) × 100.
In relation to the dietary intake of 1.2 g and 3.0 g *t*11 both *t*11 and *c*9,*t*11 CLA concentrations increased significantly in serum lipids and the *t*11 conversion rates were 20% and 25%, respectively (TABLE 3). The *t*11 conversion rates estimated by lipids of RBC and PBMC membranes were 19% and 18%, respectively. The estimated conversion rates were almost independent of the supplemented dosage. Furthermore, in the present studies the serum and membrane lipid portion of *t*11 did not exceed the mean value of 0.5% of Σ FAME (TABLE 3).

In the calculation of *t*11 conversion rate of RBC lipids a mean *c*9,*t*11 CLA decrease of 0.07% of Σ FAME (Δ 0.07) was included as a correction factor to consider the decrease of *c*9,*t*11 CLA in all control subjects (Chapter 2, Kuhnt *et al.* 2000*a*). Furthermore, the decrease of *c*9,*t*11 CLA substantiates the effectiveness of basal diet and the good compliance of the control subjects. The conversion rate of *t*11 in RBC membranes would be 9% without the correction factor compared to 19% confirmed by conversion rates of serum (20-25%) and of PBMC (18%, TABLE 3).

TABLE 3

Comparison of lipid concentrations of supplemented *t*11 and *t*12 and their Δ9-desaturation products *c*9,*t*11 CLA and *c*9,*t*12 18:2 of test groups of both present studies.

			Pre-study		**Main study**					
Test subjects			6 ♀		6 ♂, 6 ♀					
Duration [d]			28		42					
Dose [g/d]			Σ2.4 *t*11/*t*12		Σ6.0 *t*11/*t*12					
Lipids			**Serum**		**Serum**		**RBC**		**PBMC**	
			I	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Supplemented substrates 18:1 [% of ∑ FAME]	***t*11**	♀	**0.10**	**0.51**	0.07	0.52	0.09	0.46	0.07	0.49
		♂	/		0.08	0.51	0.09	0.42	0.03	0.40
		M			**0.07**	**0.52**	**0.09**	**0.43**	**0.05**	**0.45**
	***t*12**	♀	**0.08**	**0.69**	0.07	0.89	0.12	0.90	0.00	0.93
		♂	/		0.06	0.80	0.10	0.84	0.00	0.74
		M			**0.07**	**0.84**	**0.10**	**0.87**	**0.00**	**0.84**
Δ9-desaturation products 18:2 [% of ∑ FAME]	***c*9,*t*11**	♀	**0.22**	**0.36**	0.17	0.35	0.16	0.20	0.09	0.17
		♂	/		0.16	0.27	0.14	0.16	0.08	0.15
		M			**0.16**	**0.32**	**0.15**	**0.18**	**0.08**	**0.16**
	***c*9,*t*12**	♀	**0.02**	**0.04**	0.01	0.00	0.07	0.06	0.08	0.08
		♂	/		0.01	0.01	0.08	0.07	0.07	0.08
		M			**0.01**	**0.01**	**0.08**	**0.07**	**0.08**	**0.07**
Conversion rate [%] (Min-Max)[1]	***t*11**	♀	**20** (14-28, one NR)		27 (14 - 40)		11* (5 - 22)		17 (8 - 28)	
		♂	/		23 (16 - 28)		7* (3 - 15)		18 (9 - 23)	
		M			**25**, one NR		**19**† **(9*)**		**18**	
	***t*12**	M	**0**				**0** (0-2)			

I, Adaptation period; II, Intervention period; ♀, women; ♂, men; M, Mean; RBC, red blood cells; PBMC, peripheral blood mononuclear cells; NR, non-responder. [1]Non-responder are not included, *without the correction factor Δ 0.07, †with the correction factor Δ 0.07.

The *c*9,*t*11 CLA content of control PBMC lipids did not decrease that much (-0.01% of Σ FAME; Chapter 4, Kuhnt *et al.* 2007) during intervention period compared to *c*9,*t*11 CLA of lipids of RBC membranes. However, this could be a result of the generally lower baseline concentrations of *c*9,*t*11 CLA in PBMC membranes. These differences between lipid concentration of RBC and PBMC could be caused by differences of their metabolism, turn

over, and different life spans (e.g., RBC approximately 120 d; lymphocytes from several days to a lifetime as memory lymphocytes).

Presumably, the two-wk adaptation period was not adequate to adapt to the basal diet and to ´wash out´ these fatty acids (Moore *et al.* 1980). However, to adjust the RBC conversion rate the *c*9,*t*11 CLA decrease observed in control RBC membranes the correction factor Δ 0.07 was used for the calculation (Chapter 2, Kuhnt *et al.* 2000*a*).

Overall, the baseline concentrations of *t*11 and *t*12 were comparable in all analyzed lipids in both studies. Despite the 1:1 supplementation of both *trans* isomers the *t*11 content of serum and membrane lipids was approximately 50% lower compared to *t*11 (*t*11 0.5%, *t*12 0.9% of Σ FAME, TABLE 3). The *t*12 Δ9-desaturation product *c*9,*t*12 18:2 remained unchanged in analyzed lipids, which indicates that *t*12 is no adequate substrate for Δ9-desaturation in humans. Due to the unchanged *c*9,*t*12 18:2 content the *t*12 conversion rate was estimated to be 0%. No change of *c*9,*t*12 18:2 in lipids of control samples was detected as well (TABLE 3).

In rats fed *t*11 and *t*12 [1:1] similar results were shown. The *t*12 was Δ9-desaturated into *c*9,*t*12 18:2 but importantly lower than *t*11 into *c*9,*t*11 CLA (Kraft *et al.* 2006*b*). The mean *t*12 conversion rate was generally low at 2% while the conversion rate of *t*11 was approximately ten-fold higher (Kraft *et al.* 2006*b*). In cows the conversion of *t*12 was higher than observed in rats and humans, but rather lower than of *t*11 (Griinari *et al.* 2000). In the present studies, in few subjects a slight increase of *c*9,*t*12 18:2, however, could be detected as well (Δ 0.01 of Σ FAME). This indicates in some subjects a low *t*12 conversion of 1 to 2% (TABLE 3). The very marginal increase of *c*9,*t*12 18:2 was at least not significant and could be a result of variations in the analytical methods as well.

The cause for the observed differences in metabolism of *t*11 and *t*12 is still unknown. Firstly, it could be possible that the *trans*-double bond at the 12th C-atom may posses a higher steric hindrance for the Δ9-desaturase binding site than the *trans*-double bond at the 11th C-atom. Secondary, as shown for the *c*11 position it is conceivable that the *t*12 position do not fit into the binding site of the Δ9-desaturase (Brett, 1971). Further enzymes involved in the desaturation process (Acyl CoA-Synthetase, FIGURE 4) may act differently with various *trans* positions. In general, the regulation of the interaction of Δ9-desaturase and its substrate could additionally involve the melting point of the total lipid in tissues (Ntambi, 1999).

Desaturation of cis11 and cis12 18:1 isomers

The *trans*-isomer mixture used in the present study contained additionally 20% of *cis*11 18:1 (*c*11) and *cis*12 18:1 (*c*12) in equal amounts as technical by-products. During the supplementation period both *cis* isomers were increased in serum (FIGURE 7) and RBC membranes as well but no increase of their desaturation products was examined. Adlof *et al.* (2000) showed a minor presence of *c*9,*c*11 CLA and suggested a similar, but less utilized, pathway for conversion of *c*11. In rats fed *c*11 a slight increase of *c*9,*c*11 CLA was detected as well (Kraft *et al.* 2006*b*). However, a schematic representation of the Δ9-desaturase of Brett *et al.* (1971) that it is improbable for *cis*-monoenoic acids to fit into the substrate binding site (except double bonds at or beyond the Δ14 position) and therefore a direct *c*11 or *c*12 Δ9-desaturation would not be possible. In the present study, the increase of dietary *c*11 (1 g/d) was not accompanied with an increase of *c*9,*c*11 CLA (Appendix TABLE A-3). At present, the formation of *c*9,*t*11 to *c*9,*c*11 CLA from *c*11 due to shifting and isomerization of the double bond is still feasible (FIGURE 8).

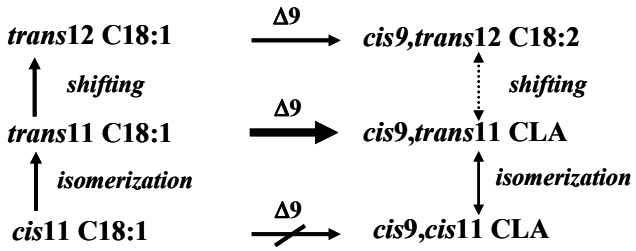

FIGURE 8 Theoretical pathways for the formation of CLA isomers from 18:1 precursors.

Individual variation of t11 conversion

The *t*11 conversion rates at average varied only marginally between different tissue lipids and supplementation periods (18 to 25%, TABLE 3). However, intra- and inter-individual differences were shown in agreement with results of Turpeinen and colleagues (2002). The individual *t*11 conversion rates showed large ranges in all analyzed lipids (TABLE 3). In serum lipids of the main study from *t*11 conversion rate ranged from 5 to 37% after 7 d and from 14 to 40% after 42 d. In a study with lactating women a wide variation of *t*11 conversion of even labeled *t*11 was observed (Mosley *et al.* 2006*b*).

In exceptional cases, test subjects showed no *c*9,*t*11 increase during the present studies. They were specified as non-responder. Non-responder were defined as subjects who showed after the *trans*-isomer supplementation despite increased *t*11 in tissue lipids, no increase of *c*9,*t*11 CLA. In the pre-study one female and in the main study one male subject were identified as

non-responder, respectively (TABLE 3). Turpeinen and colleagues (2002) identified varying conversion rates and non-responder as well.

The Δ9-desaturase is the enzyme responsible for the conversion of *t*11 to *c*9,*t*11 CLA. The Δ9-desaturase has multiple roles and is expected to affect physiological variables including differentiation, insulin sensitivity, metabolic rate, adiposity, atherosclerosis, cancer, and obesity (Ntambi *et al.* 2004). In addition, it has a crucial role in regulating the unsaturated/saturated ratio which influences membrane fluidity and signal transduction (Kim & Ntambi, 1999).

In mice, four isoforms of Δ9-desaturase (*SCD1* to *SCD4*) have been identified whereas in rats, two isoforms have been identified (*SCD1* and *SCD2*, Miyazaki *et al.* 2003; Ntambi *et al.* 2004). In humans two isoforms *hSCD1* (*SCD*) and *hSCD5* have been characterized (Zhang *et al*. 1999; Wang *et al.* 2005). *SCD* has an 85% homology to all rodents *SCD* whereas *hSCD5* shares limited homology and appears to be unique to primates (Wang *et al.* 2005). The existence of further human *SCD* is suggested. The Δ9-desaturase activity depends on various factors such as dietary factors (cholesterol, PUFA, tocopherols, and carbohydrates; Ntambi, 2004; eicosapentaenoic acid, Renaville *et al.* 2006; CLA and *t*11, Jayan & Herbein, 2000).

In the present studies, the composition of the standardized diet was almost similar for each study subject at the last wk of each study period and thus, at times of blood collection (0 d and 42 d). Therefore dietary factors can be widely excluded to explain the observed individual range of *t*11 conversion rates in the present studies.

Individual polymorphisms of the *SCD* are suspected and might be explain inter-individual variations. Genetic diversities were shown of enzymes involved in fatty acid metabolism (Galluzzi *et al.* 2001). There are indications that *PPAR* polymorphism may contribute to inter-individual variability in serum lipoprotein and lipid response during dietary intervention (Paradis *et al.* 2005). Unfortunately, during this study the examination of *SCD* polymorphisms was not conducted, however, has to be considered in further experiments.

Gender-specific variations

Gender (Lee *et al.* 1996), hormones (e.g., leptin; Cohen & Friedman, 2004), and body fat mass (Jones *et al.* 1996) can influence the Δ9-desaturase activity as well. The conversion rates of *t*11 clearly differed between genders in rats (Kraft *et al.* 2006*b*). In the recent main study, women showed generally higher *c*9,*t*11 CLA synthesis and *t*11 conversion rates (TABLE 3). This difference was significant after the first-wk intervention period (Chapter 2, Kuhnt *et al.* 2006*a*). In monocytes of female test subjects the expression of *SCD* was not increased despite

*t*11 Δ9-desaturation (Chapter 5, Kuhnt *et al.*). Cohen and Friedman (2004) reported that the isoform SCD1 expression and activity in mice were repressed by leptin. In the present study, leptin concentration and body fat mass were positively correlated in both genders (Chapter 4, Kuhnt *et al.* 2007). Both leptin and body fat mass were significantly higher in women but were not related to the *t*11 conversion rate, neither in men nor in women (TABLE 4, data not shown).

In the present main study, gender-based differences regarding the lipid peroxidation and gene expression were found (TABLE 4). Mittendorfer (2005) affirmed gender-related differences in lipid metabolism. On the one hand, these differences could be result from genetic heterogeneity (gene expression, polymorphisms). On the other hand, the endocrine milieu could responsible for observed differences (sexual hormones). Furthermore, gender variations in adipose tissue lipolysis and insulin concentration (not examined in the present study) could cause sexual dimorphic metabolism (Mauriège *et al.* 1999).

TABLE 4

Gender-specific differences observed during the dietary intervention with 6.0 g *t*11/*t*12.

Main study	Finding
Kuhnt *et al.* 2006*a* [Chapter 2]	women: higher *t*11 conversion rate
Kuhnt *et al.* 2006*b* [Chapter 3]	women: higher excretion of 8-iso-$PGF_{2\alpha}$
Kuhnt *et al.* 2007 [Chapter 4]	women: higher leptin, lower adiponectin concentrations
Kuhnt *et al.* [Chapter 5]	women: increased *COX2* and *FAT* expression men: elevated *PPARγ*, *GLUT4,* and *PBE* expression

COX, cyclooxygenase; GLUT, glucose transporter; FAT, fatty acid translocase; PBE, peroxisomal bifunctional enzyme; PPAR, peroxisome proliferator-activated receptors.

Actually, in the present study the gene response to the *t*11/*t*12 intervention clearly differed between male and female subjects (TABLE 4; Chapter 5, Kuhnt *et al.*). In this study, higher leptin levels of women could be influenced the metabolic response to fatty acid supplementation. Any gender-specific outcomes approved the exigency to examine both genders during intervention studies (Chapter 5, Kuhnt *et al.*).

Distribution of the Δ9-desaturase and their activity

The expression of *SCD* isoforms in rats and mice varies among tissues (Miyazaki *et al.* 2003, Ntambi *et al.* 2004). The distribution of Δ9-desaturase activity in humans is still unknown but

a tissue dependent distribution of the Δ9-desaturase is suggested as well (Zhang *et al.* 2005). The extent of *t*11 desaturation to *c*9,*t*11 CLA differed among tissues in animals (Palmquist *et al.* 2005, Kraft *et al.* 2006*b*). Greater differences of Δ9-desaturation between tissues could reflect a various contribution of membrane lipids in total fatty acid content of each tissue. However, in human intervention studies it is generally difficult to research tissue lipids. The present studies evaluated the fatty acids of serum and membranes of RBC and PBMC as adequate non-invasive biomarker of short- and long-term dietary fat intake. The results of PBMC lipid analysis almost confirm the observation in lipids of RBC membranes (Chapter 4, Kuhnt *et al*. 2007).

The activity of Δ9-desaturase could be generally interpreted using Δ9-desaturation indices (ratio of substrate and product; *c*9 16:1/16:0, *c*9 18:1/18:0; Attie *et al.* 2002). In 290 healthy humans the 16:1/16:0 and 18:1/18:0 ratios of adipose tissue were significantly positively correlated with mRNA concentration of Δ9-desaturase. The ratios from adipose tissue were positively correlated with serum ratios as well (Fisher, unpublished observations, 2006). In the present studies, the serum ratios of test subjects were unchanged and did not differ between the treatment groups (Chapter 2, Kuhnt *et al*. 2006*a*). These observations were confirmed by the unchanged *SCD* expression in monocytes of all test subjects (Chapter 5, Kuhnt *et al.*). However, the gene expression was analyzed in monocytes. This can not reflect the gene expression and metabolism of the whole body. Particularly, PUFA have shown to be able to decrease the *SCD* expression in adipocytes (Sessler *et al.* 1996). Due to the intake of a standardized diet during blood sampling the intake of fatty acids during both periods remained unchanged. The observed changes in gene regulation by dietary factors can only partly explain the observed changes (Chapter 5, Kuhnt *et al.*).

Site and measurement of Δ9-desaturation in humans

In lactating dairy cattles the endogenous conversion of dietary *t*11 (^{13}C-labelled) to *c*9,*t*11 CLA was confirmed with the mammary gland being the primary site of Δ9-desaturase activity. Approximately 80% of milk fat *c*9,*t*11 CLA has originated from *t*11 (Mosley *et al.* 2006*a*). In contrast, in lactating women only 10% of *c*9,*t*11 CLA in milk was endogenously synthesized from ^{13}C-labelled *t*11 (Mosley *et al.* 2006*b*). Considering this low conversion rate the majority of Δ9-desaturase activity in the mammary gland is uncertain (Mosley *et al.* 2006*b*). Turpeinen *et al.* (2002) conclude from the principal role for fatty acid synthesis the liver has presumably the highest Δ9-desaturase activity in humans. Loor and colleagues (2002) suggest, however, not liver but adipose tissue is the major site for the bioconversion of

*c*9,*t*11 CLA from *t*11 in mice. Palmquist and Santora (1999) confirmed this suggestion. In the present studies, no adipose tissue samples of test subjects were available to measure Δ9-desaturase activity. The body fat mass was not related to *t*11 conversion rates. In monocytes of women, the *SCD* expression was not higher compared to men (Chapter 5, Kuhnt *et al.*).

Research of fatty acid desaturation *in vivo* using stable isotope labeling has been documented in humans. Rhee *et al.* (1997) observed that the ^{13}C-labeled stearic acid was Δ9-desaturated at 14%. The conversion from *t*11 to *c*9,*t*11 CLA has been noted directly in only one human male subject (Adlof *et al.* 2000). In the reanalysis of data from 1978 in the serum lipids of a subject who consumed ^{2}H labeled *t*11 a 30% enrichment of ^{2}H in *c*9,*t*11 CLA was found (Adlof *et al.* 2000). In lactating women with intake of ^{13}C-labeled *t*11 the ^{13}C *c*9,*t*11 CLA was increased in milk and serum lipids with a *t*11 conversion rate of 10% (Mosley *et al.* 2006*b*).

However, the indirect measurement allowed the determination of fatty acid conversion rates as well. Intake of unlabeled *t*FA (including *t*11) showed a 30% increase of serum CLA in humans (Salminen *et al.* 1998). Furthermore, when humans consumed a diet enriched with unlabeled *t*11 the conversion was demonstrated indirectly from the measurement of increased *c*9,*t*11 CLA in lipids of serum and VLDL (Turpeinen *et al.* 2002). In the current pre- and main studies, the conversion rates were determined indirectly using unlabeled *trans* isomers as well. However, for improvement the accuracy of the indirectly measurement of the *t*11 conversion rate the diet had to be under CLA- and *t*FA-free conditions for minimizing food derived *t*11 and *c*9,*t*11 CLA in human tissues during the studies. The indirect calculation method is adequate for evaluation especially *trans* isomer conversion rates because *trans*-double bonds can not synthesized in the human body.

The mean *t*11 conversion rate investigated by Turpeinen *et al.* (2002) at three different dosages was at average 19% confirming the recent conversion rates from the pre- and main studies of 20% and 25%, respectively (Chapter 2, Kuhnt *et al*. 2006*a*). Unfortunately, due to serum´s function as transport medium the indirect calculation method of fatty acids conversion rate with serum fatty acids can not distinguish from released body stored and endogenously converted fatty acids. However, the indirect calculated *t*11 conversion rate of total rat body was similar to the *t*11 conversion rate of serum in rats (Kraft *et al.* 2006*b*).

Distribution of conjugated linolenic acids in human lipids

The percentages of individual CLA isomers of the Σ CLA content were analyzed using the silver-ion (Ag^{+})-HPLC method. For an optimal separation of CLA isomers three coupled Ag^{+}-impregnated columns (ChomSpher 5 Lipids) were used (Chapter 2, Kuhnt *et al.* 2006*a*).

In ruminant-derived food products the most abundant isomer with up to 90% is the *c*9,*t*11 CLA. Similarly, in the presented studies at baseline in determined lipids the *c*9,*t*11 CLA was the major isomer (serum 76%, RBC 78%, feces 59% of Σ CLA; FIGURE 9; Appendix TABLES 2 to 5).

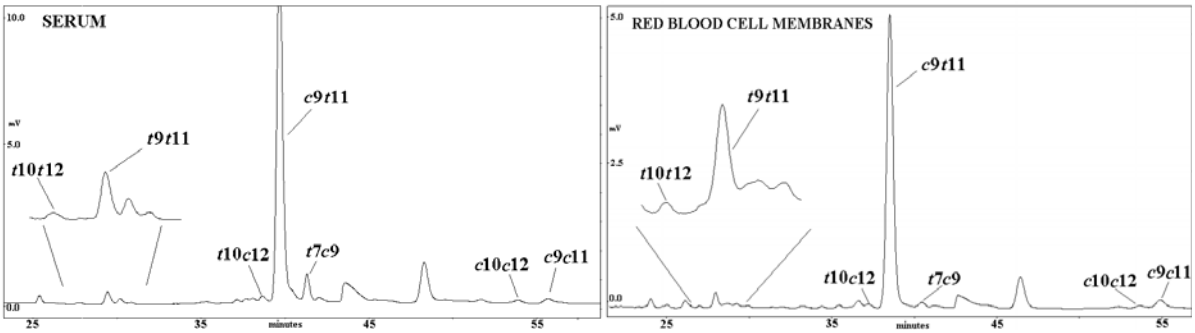

FIGURE 9 Distribution of CLA isomers of lipids in serum and red blood cell membranes of a study subject after the adaptation period [% of Σ CLA].

During the intervention period due to the *t*11 conversion the *c*9,*t*11 CLA increased in blood lipids with a maximum of 85% of Σ CLA at the expense of the *t*7,*c*9 isomer (Chapter 2, Kuhnt *et al.* 2006*a*). In lipids of feces a manifold isomeric pattern of CLA was shown, especially a high content of *t*/*t* isomers (24% of Σ CLA). This could be a result of the enzyme capacity of the colonic microbiota. Human-derived *Bifidobacterium* species and *Lactobacillus acidophilus* and *casei* were shown to be able to convert free linoleic acid primarily to *c*9,*t*11 CLA, with substantially smaller amounts of *t*10,*c*12 CLA and *t*9,*t*11 CLA (Coakley *et al.* 2003). The absorption of fatty acids occurs preferentially in the small intestine. CLA isomers formed by colonic microbiota in humans may still act locally on the colonic mucous membrane.

Trans fatty acids and conjugated linoleic acids – Effects on oxidative stress

In a broader definition, lipid peroxidation comprising any oxidation of fatty acids occurs *in vivo*. Arachidonic acid oxidation by two mechanisms is important because their primary products of two pathways are biologically active compounds. Free radical mediated non-enzymatic and COX-mediated arachidonic products, namely isoprostanes and prostaglandins, respectively, are a result of endogenous lipid peroxidation. Very little is known about the link between free radical non-enzymatic and COX-mediated pathways (FIGURE 10; Basu, 2003).

The isoprostane 8-iso-$PGF_{2\alpha}$ is called the gold standard for the evaluation of oxidative stress *in vivo*. In the present main study, the urinary 8-iso-$PGF_{2\alpha}$ concentration was increased after *t*11/*t*12 supplementation (Chapter 3, Kuhnt *et al.* 2006*b*). In a different intervention study, subjects received the same *trans*-isomer mixture as used in the present study and showed an increase of urinary 8-iso-$PGF_{2\alpha}$ as well (Turpeinen *et al.* 2002). In both studies 15-keto-dihydro-$PGF_{2\alpha}$, the biomarker of COX induced lipid peroxidation was unchanged. In studies with CLA supplementation the elevation of even both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ was shown (Basu *et al.* 2000*a,b;* Risérus *et al.* 2004, TABLE 5). Especially, the supplementation of synthetic CLA, the *t*10,*c*12 is made responsible for the observed elevations in the different CLA studies (Risérus *et al.* 2004). However, the used CLA dosages were generally higher than due to the endogenous *c*9,*t*11 CLA synthesis with *t*11 as precursor (approximately 0.7 g *c*9,*t*11 CLA/d) in the present main study.

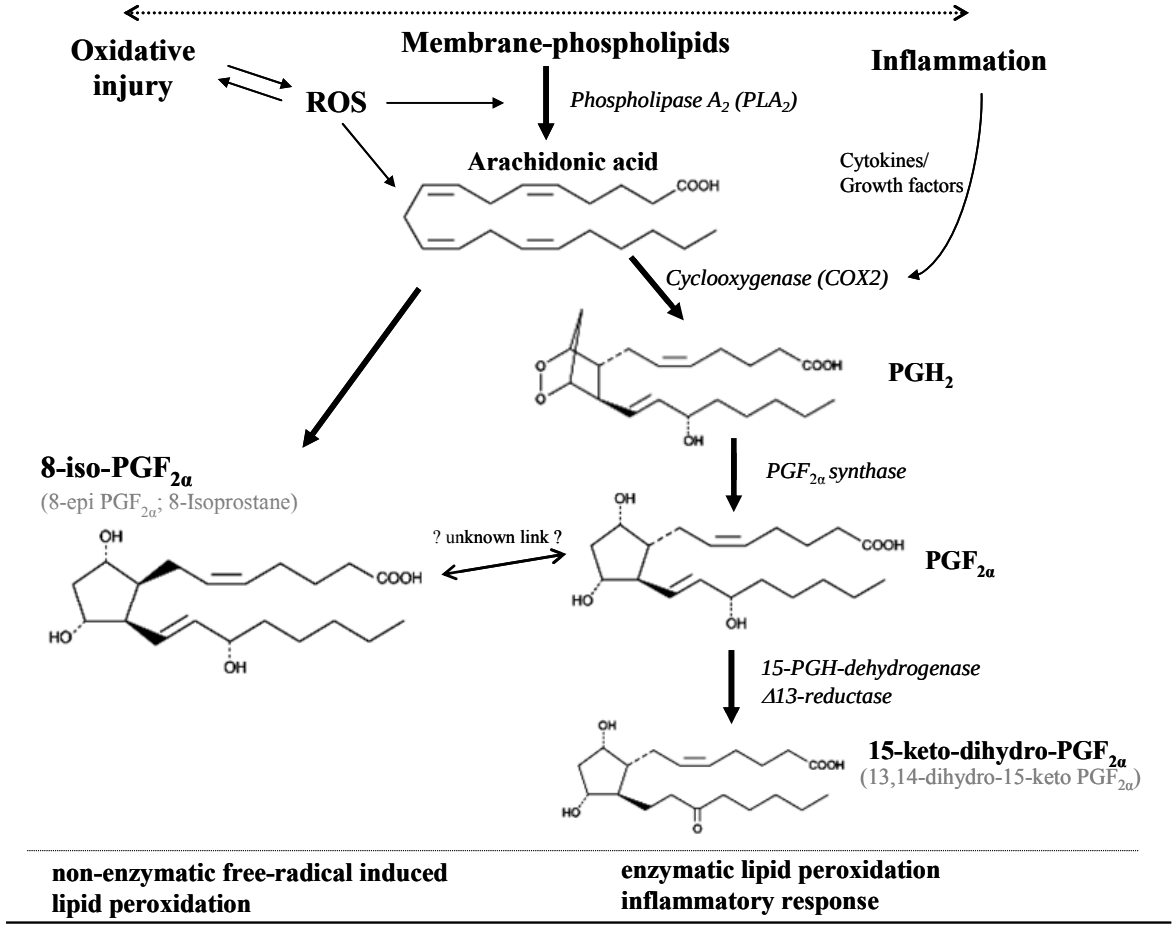

FIGURE 10 Relationship between inflammation and oxidative injury, and endogenous formation of 8-iso-$PGF_{2\alpha}$ through free-radical and $PGF_{2\alpha}$ by cyclooxygenase catalyzed oxidation of arachidonic acid. ROS, reactive oxygen species; PG, prostaglandin (modified from Basu, 2003).

In humans during the daily intake of 3.6 g *t*11 and 1.5 g *c*9,*t*11 CLA the urinary excretion of 8-iso-$PGF_{2\alpha}$ was unexpectingly unchanged (Tholstrup *et al.* 2006, TABLE 5). On the one hand, in this study *t*11 and *c*9,*t*11 CLA were administered in butter. Therefore, other butter components could supress lipid peroxidation. On the other hand, only men participated in Tholstrup´s study, which could have potentially lower 8-iso-$PGF_{2\alpha}$ formation and excretion. In the present main study (Chapter 3, Kuhnt *et al.* 2006*b*) in female subjects the 8-iso-$PGF_{2\alpha}$ urine concentration was higher than in men. Concurrently, the expression of *COX2* in female monocytes was significantly increased and therefore, identified as marker gene for *t*11/*t*12 intake (Chapter 5, Kuhnt *et al.*). The increased *COX2* expression in women indicates generally higher oxidative stress following the *t*11/*t*12 intake in women. Since we observed the induction of *COX2* and the increase of 8-iso-$PGF_{2\alpha}$ with unaffected 15-keto-dihydro-$PGF_{2\alpha}$ levels support the earlier hypothesis of Basu (2003) that the activated COX2 could be result the endogenous synthesis of 8-iso-$PGF_{2\alpha}$ may be due to enhanced $PGF_{2\alpha}$ (FIGURE 10, unknown link).

This supports the classification of the 8-iso-$PGF_{2\alpha}$ as a more sensitive marker for moderatley increased oxidative stress as in Turpeinen´s study (2002) only 8-iso-$PGF_{2\alpha}$ was increased following the *trans*-isomer intake as well. In addition, the coeval supplementation of *t*12 could be responsible for the elevated 8-iso-$PGF_{2\alpha}$ excretion compared to Tholstrup´s study. The *t*12 was supplemented in same amounts as the *t*11 (Turpeinen *et al.* 2002, main study, TABLE 5).

TABLE 5

Human studies concerning CLA and *t*FA supplementation and oxidative stress.

	Basu *et al.* 2000*a*	**Basu** *et al.* 2000*b*	**Risérus** *et al.* 2004	**Turpeinen** *et al.* 2002	**Tholstrup** *et al.* 2006	**Kuhnt** *et al.* 2006*b*
Subjects	24 ♂ (obese)	28 ♂/♀ (healthy)	25 ♂ (obese)	30 ♂/♀ (healthy)	42 ♂ (healthy)	24 ♂/♀ (healthy)
Duration	1 month	3 months	3 months	9 days	35 days	42 days
Supplements (g/d)	CLA 4.2[2]	CLA 4.2[2]	*c*9,*t*11 CLA 3.0[3]	*t*11 1.5, 3.0, 4.5[4]	*t*11 3.6 *c*9,*t*11CLA 1.5	*t*11/*t*12 6.0[4]
Increase (Δ) 8-iso-$PGF_{2\alpha}$[1]	0.81†	1.15†	0.10†	0.18†, 0.22†, 0.22†	0.0	0.10†
Increase (Δ) 15-kd-$PGF_{2\alpha}$[1]	0.21†	0.60†	0.05†	no data	no data	0.0

♂, men; ♀, women; Δ, change from baseline to intervention period. 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; 15-kd-$PGF_{2\alpha}$, 15-keto-dihydro-prostaglandin $F_{2\alpha}$. [1]Urinary concentration [nmol/mmol creatinine]. [2]*c*9,*t*11 CLA/*t*10,*c*12 CLA, 1:1. [3]83% *c*9,*t*11 CLA & 7% *t*10,*c*12 CLA. [4]*trans*-isomer mixture (*t*11/*t*12, 1:1; Natural ASA). †Significantly different to baseline and to their -if available- corresponding control group ($P \leq 0.05$).

Trans fatty acids and conjugated linoleic acids – Effects on serum lipids and lipoproteins

*t*FA intake is mainly associated with changes of the serum lipid profile (Ascherio, 2006; Zock & Katan, 1997). The effects of *t*11/*t*12 on human serum lipids exclusively were not studied before. In the present studies with daily 2.4 g and 6.0 g *t*11/*t*12 over 28 and 42 d, respectively, no differences between serum lipoproteins, cholesterol, and TAG concentrations of the treatment groups could be determined (TABLE 6). Neither the ratio of total cholesterol to HDL cholesterol, a powerful predictor of coronary heart disease (Stampfer *et al.* 1991) nor the commonly used ratio of LDL cholesterol to HDL cholesterol was changed (TABLE 6).

The recent study of Tholstrup *et al.* (2006) confirmed that the supplementation with daily pure 3.6 g *t*11/d as a comparable dose like in the present main study had no effects on serum lipids. On that account, the effect of various *t*FA on blood lipids could be isomer specific and had to be examined separately in further studies.

TABLE 6

Serum lipids and lipoprotein profile after *t*11/*t*12 supplementation with 2.4 g/d and 6.0 g/d.

	Intervention periods[1]			
	Σ 2.4 g *t*11/*t*12, day 28[†]		Σ 6 g *t*11/*t*12, day 42[2,††]	
	Control group (*n* = 6)	**Test group** (*n* = 6)	**Control group** (*n* = 12)	**Test group** (*n* = 12)
TAG [mmol/L]	0.9 ± 0.3	0.7 ± 0.2	0.9 ± 0.3	0.9 ± 0.3
Total C [mmol/L]	4.6 ± 0.7	4.7 ± 1.1	4.2 ± 0.4	4.4 ± 0.8
HDL C [mmol/L]	1.6 ± 0.5	1.7 ± 0.9	1.4 ± 0.2	1.4 ± 0.4
LDL C [mmol/L]	2.8 ± 0.8	2.5 ± 0.6	2.4 ± 0.6	2.7 ± 0.8
LDL C/HDL C	1.8 ± 0.7	1.5 ± 0.8	1.9 ± 0.6	2.1 ± 0.7
Total C/HDL C	2.9 ± 0.6	2.8 ± 1.0	3.2 ± 0.5	3.4 ± 0.7

TAG, triacylglycerols; C, cholesterol. [1]no differences between the treatment groups with baseline as covariate. [2]no sex × treatment interaction . [†]only women, [††]women (*n* = 6) and men (*n* = 6) subjects.

For example, since pure CLA isomers are available isomeric specific differences are more pronounced. The *c*9,*t*11 CLA decreased LDL to HDL cholesterol and total to HDL cholesterol, whereas the *t*10,*c*12 CLA showed opposite effects and increased them (Tricon *et al.* 2004). A recent study of this work group found that dairy products naturally enriched with *c*9,*t*11 CLA (1.4 g/d), however, and the high dosage of their precursor *t*11 (4.7 g/d) have no significant effects on the serum lipid profile as well (Tricon *et al.* 2006). In contrast, the *t*9 (5.5 en%) appeared to affect the lipoprotein profile relative to specific SFA in humans (Sundram *et al.* 1997).

At present, it is more clearly that there are different metabolic properties of different *t*FA and CLA isomers. Their distribution is probably strongly associated with the dietary source and thus, with there isomeric pattern.

Source and trans fatty acid profile – clinical relevance

In ruminant fats including milk and cheese the *t*11 is the predominant *trans* 18:1 isomer accompanied with ruminant-derived CLA isomers, whereas industrially hydrogenated oils and fats contain various *trans* isomers, especially *t*9, followed by *t*6/7, *t*10, *t*11, *t*12 and have only marginal amounts of CLA. Maybe therefore, the association of cardiovascular disease with the intake of *t*FA from ruminant fats is less consistent than does the intake of partially hydrogenated oils and fats (Mensink *et al.* 2003, Willet *et al.* 1993). It is interesting to note that the Danish Nutrition Council concluded that, "the available data indicates that *t*FA from ruminants, particularly so far as the risk of heart disease is concerned, do not have the same adverse effects as industrially produced *t*FA" (Danish Ministry of Food, Agriculture and Fisheries, 1997). In contrast, Oomen *et al.* (2001) found similar non-significant direct associations between both industrially produced and ruminant *t*FA and the risk of cardiovascular disease. The available data imply that the association between the two sources of *t*FA and risk of cardiovascular disease were described across different ranges of intake. Weggemans *et al.* (2004) concluded that the intake of up to 2.5 g *t*FA/d show no differences for ruminant and industrial *t*FA. At higher intakes, both total *t*FA and industrially produced *t*FA were associated with an increased risk, whereas at present insufficient data for evaluation of ruminant *t*FA are available (Weggemans *et al.* 2004). Furthermore, it is important to note, that not only the *trans*-isomer profile but the consumed amounts of total fat and *trans* fat is decisive for health risks. The industrially processed foods are commonly high in fat.

The Danish population is adequate to research the effects of dietary ruminant fats. Since in Denmark the intake of industrially produced *t*FA has been lowered from 2.2 g/d in 1992 to 0.35 g/d in 1999 (Leth *et al.* 2003), whereby the intake of ruminant *t*FA stayed consistently at 1.8 g/d (Jakobsen *et al.* 2006*a*). In their recent prospective cohort study it was concluded that the intake of ruminant *t*FA with *t*11 as major *trans* isomer is innocuous and is not associated with higher risk of cardiovascular disease (Jakobsen *et al.* 2006*b*). The TRANSFACT study – a controlled cross-over study – is the first study comparing the intake of *t*FA of both industrial (major *t*9) and ruminant (major *t*11) in humans (Chardigny *et al.* 2006*a*). Different effects on serum lipids and lipoproteins were shown between both *t*FA sources. The industrially produced *t*FA decreased the HDL, VLDL, and LDL cholesterol concentrations compared to

the ruminant *t*FA. The treatment effects were significant only in women (Chardigny *et al.* 2006*b*). This demonstrates both gender- and source-related effects of *t*FA and had to be involved in the assessment of risk. Furthermore, other studies will have to involve subgroups of the population (gender, age) in the future.

Trans fatty acids and conjugated linoleic acids in tissue lipids – clinical relevance

Tissue *t*FA are adequate biomarkers for *t*FA intake (Chapter 2, Kuhnt *et al.* 2006*a*). Since the straighter configuration of *trans* relative to *cis* isomers resembles that of SFA and are able to replace *cis* fatty acids and can change biological functions of cell membranes. In most of the case-control studies *t*FA concentrations of serum lipids and cell membranes were positively correlated to cardiovascular disease in humans (TABLE 7). Two studies do not support that *t*FA increase the risk of cardiovascular disease (Roberts *et al.* 1995, Fritsche *et al.* 1998). Aro et al. (1995) analyzed only total *trans* 18:1 isomers and found no overall effect on risk of myocardial infarction. There is strong evidence that single *trans* 18:1 isomers differ in their potency to induce cardiovascular disease. For example, in a cross sectional study of patients, who had angiographically documented coronary heart disease the *t*9 and *t*10 isomers of platelet lipids were positively associated with the extend score of coronary heart disease (Hodgson *et al.* 1996). The *t*12 showed a trend of association ($P = 0.058$) to the extent score. Interestingly, in contrast the *t*11 was not correlated (Hodgson *et al.* 1996).

In the present studies, the *t*12 was significantly higher in membrane lipids of RBC and PBMC than *t*11 with and without *t*11 conversion (TABLE 3). Regarding to the possible competitive inhibition of the incorporation of *cis* fatty acids the *t*12 could be more risky to have detrimental effects on membrane functions due to its higher incorporation. The *t*9 isomer is self-evident no substrate of Δ9-desaturase. The *t*8 and *t*10 did not give *t*8, *t*9 or *t*9, *t*10-allenes (Pollard, 1980). The Δ9-desaturation of *t*11 to *c*9,*t*11 CLA contributes an advantage when compared to other *trans* 18:1 isomers such as *t*9, *t*10 and as shown to *t*12.

James *et al.* (2006) confirmed that fatty acids of RBC membranes of patients with coronary artery disease were highly correlated with fatty acids of myocardial phospholipids. Studies in humans (Lemaitre *et al*. 2002, 2006; Baylin *et al.* 2003) explicitly showed a positive association of *trans* 18:2 and not of *trans* 18:1 in RBC membranes and plasma phospholipids to primary cardiac arrest and sudden cardiac death, respectively (TABLE 7). The authors defined them as mainly *trans* 18:2 isomers from industrially produced fats and do not include the naturally derived CLA isomers. Furthermore, the differentiation of single *trans* isomers is still absent.

Several clinical studies indicate that dietary fatty acids influence cardiac arrest and arrhythmias (Katz, 2002). Finally, pro- and anti-arrhythmic effects depend on interactions between fatty acids and the bilayer structure. Moreover, membrane fluidity and function, for example, the opening, closing, and inactivation of ion channels could be changed due to increased incorporation of *trans* isomers (Katz, 2002).

The *t*9 and *t*9,*t*12 18:2 isomers increase the calcium influx into human arterial endothelial cells (Kummerow *et al.* 1999). This could enhance stenosis characterized by lipid and calcium deposits in the arterial wall. Stachowska *et al.* (2004*b*) found in atheromatous plaques and adipose tissue of patients with atherosclerotic stenosis the *t*9 and *t*10 with the highest concentrations. In general, the results of the case-control studies nevertheless varied by different analytical methods and differences among tissue lipids.

TABLE 7

Case-control studies – *t*FA concentration in tissue lipids and risk of cardiovascular disease.

Case-Control Study	Tissue	Cases	*Trans* isomers	Correlation ($P \leq 0.05$)
Thomas *et al.* (1983)	Adipose tissue	Ischemic heart disease	*t*16:1, *t*18:1	+
			*t*20 and *t*22	/
Siguel & Lerman (1993)	Plasma	Coronary heart disease[†]	total *t*FA; *t*11 16:1	+
			*t*9,*t*12 18:2	+
			*t*9 18:1; *t*11 18:1	/
Aro *et al.* (1995)	Adipose tissue	Myocardial infarction	*t*18:1	/
Roberts *et al.* (1995)	Adipose tissue	Sudden cardiac death	total *t*FA, *t*18:1	–
			*t*18:2	/
Hodgson *et al.* (1996)	Platelets	Coronary heart disease[†]	*t*9, *t*10	+
			*t*9 16:1; *t*11, *t*12[‡]	/
			*t*9,*t*12 18:2	/
Fritsche *et al.* (1998)	Adipose tissue Plasma lipids	Coronary heart disease[†]	total *t*FA	/
Lemaitre *et al.* (2002)	Red blood cell PL	Primary cardiac arrest	total *t*FA, *t*18:2	+
			*t*18:1	/
Baylin *et al.* (2003)	Adipose tissue	Nonfatal myocardial infarction	total *t*FA, *t*16:1, *t*18:2	+
			*t*18:1	/
Dlouhý *et al.* (2003)	Subcutaneous fat	Coronary heart disease[†]	total *t*FA	/
			*t*18:1	+
Clifton *et al.* (2004)	Adipose tissue	Myocard. infarction	*t*9, *t*10, *t*11	+[*]
Lemaitre *et al.* (2006)	Plasma PL	Fatal ischemic heart disease, sudden cardiac death	total *t*FA, *t*16:1	–
			*t*18:1 (6/8, 9, 10, 11, 12)	–
			*t*18:2 (9,12; *c*,*t*; *t*,*c*; *t*,*t*)	+

–, cases lower than controls; +, cases higher than controls; /, cases not different to controls. *t*FA, *trans* fatty acids; PL, phospholipids. [†]Angiographically documented stenosis in coronary vessel, [‡] ($P = 0.058$), [*]after 1996 – no differences between cases and controls.

A further point of criticism of the studies is the missing and unclear determination of CLA. CLA concentrations in tissue lipids are even associated with anti-inflammatory and anti-atherogenic effects, however, at present significantly only in animals. The natural *c*9,*t*11 CLA in the diet reduced the outcome of atherogenic process in hyperlipidemic hamsters (Valeille *et al.* 2006).

CLA compete with arachidonic acid for access to enzymes, therefore preventing its metabolism to eicosanoids. Consequently, CLA inhibit platelet aggregation and PG formation in macrophages (Yu *et al.* 2002). However, *t*FA are thought to inhibit fatty acid desaturation and incorporation in membranes as well (Stachowska *et al.* 2004*a*). Present data showed the decrease of arachidonic acid (Chapter 2, Kuhnt *et al.* 2006*a*; Chapter 4, Kuhnt *et al.* 2007) as well as of further long chain fatty acids such as 22:6 *n*-3 in cell membrane lipids (Chapter 4, Kuhnt *et al.* 2007). However, arachidonic acid portion of lipids of control subjects was decreased as well. No changes of $sPLA_2$ activity and PGI_2 concentrations have been found in both treatment groups. Further eicosanoids derived from COX and LOX were unfortunately not determined during this study.

Toomey *et al.* (2006) summarized that CLA induces resolution of atherosclerosis by negatively regulating the expression of pro-inflammatory genes and inducing apoptosis in the atherosclerotic lesion in mice. CLA decreased the production of pro-inflammatory products in macrophages and smooth muscle cells basically due to PPARγ dependent mechanisms (Yu *et al.* 2002, Ringseis *et al*. 2006). CLA have been shown to activate PPARα as well (Benjamin *et al.* 2005). A recent study shows that both PPARα and γ activators inhibit foam cell formation *in vivo* (Li *et al.* 2004). However, the underlying mechanisms of action are still poorly understood. In the present main study, *PPARα, β,* and *γ* expression in monocytes were analyzed. *PPARγ* showed a significantly higher expression in male test subjects than in the adaptation period. *PPARγ* and the insulin sensitive glucose transporter *GLUT4* were identified as marker genes during *t*11/*t*12 intervention in male test subjcets. Contrary, during the *t*FA- and CLA-free basal diet the *PPARγ* and *GLUT4* expression was down-regulated in male control subjects. (Chapter 5, Kuhnt *et al.*).

At present, the Δ9-desaturation appears to be the key differentiating *t*11 from other *trans* 18:1 isomers. Due to *t*11 conversion to *c*9,*t*11 CLA only the pure *c*9,*t*11 CLA is generated. The investigation of the single effect of *t*11 in the human organism is almost impossible, except in non-responder. At the moment, opposing effects of *t*11 and *c*9,*t*11 CLA can not be excluded.

The endogenous *c*9,*t*11 CLA synthesis from *t*11 was associated with anti-carcinogenic effects in animals (Lock *et al.* 2004). The *c*9,*t*11 CLA was able to potently inhibit carcinogenesis, especially of the mammary gland in animal models (Ip *et al.* 2000). The anti-carcinogenic effect of *t*11 was definitely exclusively mediated through its conversion (Banni *et al.* 2001, Corl *et al.* 2003). The relationship of *c*9,*t*11 CLA and mammary cancer protection in humans was examined through epidemiological studies and the results have been inconclusive.

Aro *et al.* (2000) found that serum *c*9,*t*11 CLA was significantly lower in breast cancer cases than in controls in postmenopausal women. The authors conclude that CLA from dairy products could have important impact as protective agent against breast cancer. The study of Voorrips *et al.* (2002) did not confirm a beneficial effect for *c*9,*t*11 CLA. Furthermore, in a case-control study no significant association between the concentration of *c*9,*t*11 CLA of breast adipose tissue and the risk of breast cancer was detected (Chajès *et al.* 2002).

Reducing the intake of trans fatty acids

Since the undesirable effects on lipoproteins of *t*FA were observed (Mensink & Katan, 1990) the food industry is working on ways to eliminate or greatly reduce them in food products. The reduction of industrially produced *t*FA in the diet could minimize their accumulation in tissues and reduce the associated risk of disease. Clifton *et al.* (2004) showed the rapid disappearance of *t*FA in adipose tissues when they were not included in margarines after June 1996 in Australians.

Current efforts focus on 4 technological options: (1) modification of the hydrogenation process, (2) use of inter-esterification, (3) use of fractions high in solids from natural oils, and (4) use of trait-enhanced oils (Hunter, 2005). Some high fat industrially processed foods such as margarines available on the market contain approximately 40% *t*FA of Σ fatty acids. In contrast, comparable food products containing only minor amounts of *t*FA (Kraft *et al.* 2006*a*, Clarke & Lewington, 2006). Palm oil and coconut oil are common alternatives for the industry (Aro, 2006). These oils are high in SFA and often lead to products that are higher in SFA and lower in unsaturated fatty acids. Therefore, the fatty acid composition of high fat processed foods is unfavorable irrespective of their *t*FA content. In general, dietary guidelines should advise not to consume high fat industrially processed foods (Aro, 2006). The consumers need to know, which products to choose. Actually, since Canada introduced the food labeling of *t*FA on retail foods *t*FA content declined in human milk suggesting a concomitant decrease of *t*FA intake among lactating women and breast-fed infants (Friesen &

Innis, 2006). Therefore in the EU, mandatory addition of SFA and *t*FA content on nutrition labels is claimed to simplify the purchase decision of the consumers.

The Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) of the European Food Safety Authority (EFSA) concluded that at equivalent dietary levels, the effect of *t*FA on heart health may be greater than that of SFA. However, current intakes of *t*FA are generally more than 10-fold lower than those of SFA whose intakes in many European countries exceed dietary recommendations (EFSA, 2004). The NDA panel concluded that evidence with regards to a possible association of *t*FA intake with cancer, diabetes mellitus type-2 or allergies is weak or inconsistent. In addition, the Australia New Zealand Food Standards Code does not require manufacturers to label the *t*FA content of foods as well (Booker & Mann, 2005).

Compared to the US and Canadian population, the *t*FA intake is indeed generally low (< 1 en%) but in some subgroups the intake can be very high. Stender *et al.* (2006) defined a so-called "high *trans* menu" as a large size serving (French fries, nuggets, 100 g of microwave popcorn, and 100 g of biscuits/wafers/cakes) consisting of approximately 30 g industrial *t*FA. According to the mandatory restriction in 2004 in Denmark the *t*FA content of the menu was reduced below 1 g. By contrast, a "high *trans* menu" provided more than 20 g in 17 of 18 countries, with Hungary, Czech Republic, Bulgaria, and USA ranking highest with 42 g/menu. These extremely high amounts of *t*FA implicate an increased risk for subgroups of the population and the requirement of mandatory regulation of *t*FA content of industrially produced oils (Stender *et al.* 2006).

In conclusion, data from epidemiological studies indicate that the consumption of *t*FA from partially hydrogenated fats provide no apparent nutritional benefit and has partly detrimental effects. Available data support an important link to the increased risk of cardiovascular disease.

On the basis of previous and present data, it is generally prudent to reduce the intake of *t*FA, especially from high-fat industrially processed products. These products contain high *t*FA amounts and have an *trans*-isomer profile with mainly *t*9 and *t*10 (18:1) assumed to have detrimental metabolic properties. Both isomers are no substrates of the Δ9-desaturase. In ruminant-derived fats the *t*11 is the major *trans* isomer. The present studies have shown due to the high dietary *t*FA intake their increased incorporation into membrane lipids. The supplemented *t*11 was identified as adequate precursor of *c*9,*t*11 CLA synthesis by Δ9-desaturation in humans. Thereby, *t*12 was not converted to the *c*9,*t*12 18:2 and the *t*12 portion

was approximately 2-fold higher in membrane lipids than *t*11. The Δ9-desaturation of *t*11 appears to be the key in differentiating the naturally derived *t*11 isomer from *t*9, *t*10, and other *trans* 18:1 isomers as currently shown for the *t*12 in the present studies. Ruminant-derived foods have generally low amounts of *t*FA based on the total milk fat content, are naturally rich in *c*9,*t*11 CLA, and have the *t*11 as major *trans* isomer. As shown in the present studies *t*11 is a potent precursor of *c*9,*t*11 CLA and contributes to the CLA supply in humans.

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SUMMARY

BACKGROUND A relationship between high *trans* fatty acids (*t*FA) intake and the risk of cardiovascular disease is assumed but most likely with differences among positional isomers of *trans*-double bonds and the type of double bonds (isolated *vs* conjugated). The *trans*-isomer distribution in foods differs depending on their source and processing. Foods containing partially hydrogenated fats and oils have partly high *t*FA content with predominantly *t*6/7/8, *t*9, *t*10, *t*11, and *t*12 octadecenoic acid (18:1). In contrast, ruminant-derived fats contain mainly the vaccenic acid (*t*11). The *t*11, endogenously formed by Δ9-desaturation in the mammary gland, is the major source of *c*9,*t*11 18:2 (conjugated linoleic acid, CLA) in milk fat. Therefore, the *t*11 conversion to *c*9,*t*11 CLA in humans could be relevant for their CLA supply. CLA exhibit various effects, such as anti-cancerogenic, anti-inflammatory, and anti-atherogenic with isomer-specific differences (*c*9,*t*11 and *t*10,*c*12).

OBJECTIVES At present, the efficiency of Δ9-desaturation of *t*11 and *t*12 to their Δ9-desaturation products *c*9,*t*11 CLA and *c*9,*t*12 18:2 during their simultaneous intake is unknown in humans. Furthermore, the incorporation of both supplemented substrates and their Δ9-desaturation products into human tissue lipids as well as their potential effects on human health are currently uncertain. For clarifying these questions two human intervention studies (pre-study and main study) were conducted.

DESIGN In both studies healthy subjects had to consume a diet, which contained marginal amounts of *t*FA and CLA (industrial and ruminant derived) during the entire studies (basal diet). The studies started with a 14-d adaptation period without supplementation to establish baseline values. In the pre-study the female test subjects received daily 2.4 g of *t*11 and *t*12 (ratio 1:1) over a 28-d intervention period. In the main study, male and female test subjects consumed daily 6.0 g/d over a 42-d intervention period. In both studies, control subjects received a *t*FA- and CLA-free control oil to balance the fat intake. In the last week of each period the subjects received a standardized diet. During this time blood, feces, and urine were sampled.

RESULTS

Fatty acid analyses According to the *t*11/*t*12 supplementation both *t*11 and *t*12 increased significantly in lipids of serum and membranes of red blood cells (RBC) and peripheral blood mononuclear cells (PBMC), each isomer at a specific amount. The *c*9,*t*11 CLA content was significantly increased in serum and membrane lipids of test subjects as well. In relation to the daily *t*11 intake of 1.2 g and 3.0 g the *t*11 conversion rate to *c*9,*t*11 CLA was 20% and 25% in serum, respectively. In contrast, the *c*9,*t*12 18:2 was not increased in the lipids. This indicates that *t*12 is not a substrate of the Δ9-desaturase. The membrane lipids of RBC have been evaluated as adequate biomarker of dietary fatty acids. During the *t*11 and CLA-free basal diet the *c*9,*t*11 CLA content decreased significantly in RBC of control subjects. Considering fatty acids of RBC of control and test subjects the *t*11 conversion rate was calculated at 19%. In lipids of PBMC membranes the *t*11 conversion rate was 18%. Considering the conversion of *t*11 the incorporated *t*12 portion of cell membranes was 2-fold higher than *t*11 (FIGURE 11). High inter- and intra-individual as well as gender-related variations of *t*11 conversion have been determined.

Gene expression Data of gene expression in monocytes of study subjects (6.0 g *t*11/*t*12) showed a gender-differentially gene regulation. In female test subjects the *cyclooxygenase 2 (COX2)* and *fatty acid translocase* (*FAT*) whereas in male test subjects the *peroxisome proliferator-activated receptor (PPAR) γ, glucose transporter (GLUT) 4,* and *peroxisomal bifunctional enzyme* were significantly increased. In male control subjects *PPARγ, GLUT1,* and *GLUT4* were significantly down-regulated. Generally high gene response was observed in control female subjects (FIGURE 11).

Clinical relevance The oxidative stress marker 8-iso-$PGF_{2\alpha}$ (non-enzymatic) was increased in the urine in relation to the *t*11/*t*12 supplementation (6.0 g/d). The 15-ketodihydro-$PGF_{2\alpha}$ and 8-oxodG – biomarker of COX2-mediated inflammatory response and oxidative DNA-damage – were unaffected, respectively. The anti-oxidative status in plasma of test subjects compared to baseline was unchanged. Serum lipids and lipoproteins, and biomarker of the immune system and inflammation (phagocytosis, interleukines, TNFα, CRP, prostacyclin, $sPLA_2$, ICAM-1, leptin, etc.) indicate no detrimental effects of dietary intake of *t*11 and *t*12 on human health (FIGURE 11).

The observed effects on gene expression and oxidative stress can be attributed to *t*11 and/or *t*12 as well as to the synthesized *c*9,*t*11 CLA. It is also possible that *c*9,*t*11 CLA might compensate detrimental effects of *t*11/*t*12 – if they have any.

CONCLUSION On the basis of previous and present data, it is generally prudent to reduce the intake of *t*FA, especially from high fat industrial processed products. These products contain partly *t*9 and *t*10 as major *t*FA supposed to have detrimental metabolic properties. Both *trans* isomers are no substrates of the Δ9-desaturase. In contrast, in ruminant-derived fats the *t*11 is the major *trans* isomer. The present studies identified the supplemented *t*11 as applicable precursor of *c*9,*t*11 CLA. The supplemented *t*12 was not converted to *c*9,*t*12 18:2 and was 2-fold higher in cell membrane lipids than *t*11. The Δ9-desaturation of *t*11 appears to be the key in differentiating *t*11 from other *trans* 18:1 isomers such as *t*9, *t*10, and as currently shown for the *t*12 in the present studies. In general, ruminant fats are naturally rich in *c*9,*t*11 CLA and *t*11. As shown in the present studies *t*11 is a potent precursor of *c*9,*t*11 CLA and contributes to the CLA supply in humans.

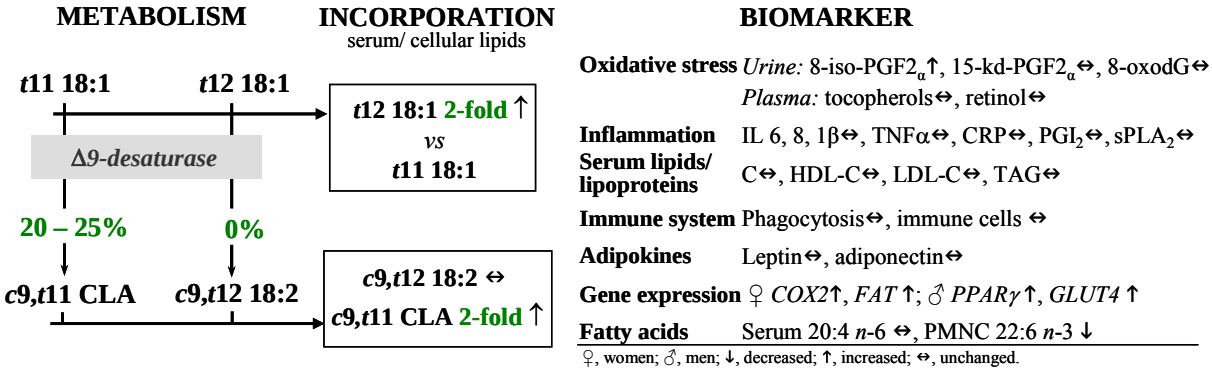

FIGURE 11 Δ9-desaturation of *t*11 and *t*12 to *c*9,*t*11 CLA and *c*9,*t*12 18:2 and the effects in humans.

ZUSAMMENFASSUNG

GRUNDLAGEN Zahlreiche Studien deuten darauf hin, dass die erhöhte Aufnahme von *trans*-Fettsäuren (*t*FS) mit einer Risikoerhöhung für kardiovaskuläre Erkrankungen assoziiert ist. Dabei scheint die Position der *trans*-Doppelbindung für die Risikobewertung relevant zu sein. Der Gehalt und die Verteilung der *t*FS in Lebensmitteln variieren mit ihrer Genese. Bei der technischen Hydrierung von Fetten und Ölen entstehen teilweise hohe Mengen *t*FS. Industriell verarbeitete Lebensmittel enthalten besonders *t*6/7/8-, *t*9-, *t*10-, *t*11- und *t*12-Octadecensäure (18:1). Durch bakterielle Biohydrogenierung von ungesättigten Fettsäuren im Pansen von Wiederkäuern entstehen ebenfalls *t*FS, mit Vaccensäure (*t*11) als vorherrschendes *trans*-Isomere. Die *t*11 wird durch die Δ9-Desaturation im Gewebe zur *c*9,*t*11 18:2 (*c*9,*t*11 CLA, Isomere der konjugierten Linolsäuren) konvertiert und stellt die Hauptquelle für CLA im Milchfett dar. Durch die endogene Δ9-Desaturation könnte *t*11 als Präkursor für die CLA-Synthese im Menschen relevant sein. CLA können sich durch ihre anti-kanzerogenen, anti-inflammatorischen und anti-atherogenen Wirkungen positiv auf die Gesundheit auswirken.

ZIELE Ziel der vorliegenden Arbeit war es, die Δ9-Desaturation von *t*11 und *t*12 (18:1) zu deren Δ9-Desaturationsprodukten *c*9,*t*11 CLA und *c*9,*t*12 18:2 im Menschen zu untersuchen. Außerdem wurde die Inkorporation beider *trans*-Isomeren und deren Konversionsprodukten in Membranlipide untersucht. Im Rahmen dieser Arbeit wurde außerdem der Einfluss der supplementierten *t*FS auf diverse Biomarker für oxidativen Stress, Inflammation und die Immunfunktion untersucht.

DESIGN In beiden Studien (Vor- und Hauptstudie) erhielten gesunde Probanden eine Basiskost, welche nur einen unbedeutenden Anteil an CLA und *t*FS (industriell und natürlich) enthielt. Beide Studien begannen mit einer 14-tägigen Adaptationsperiode ohne Supplementation, um Basiswerte zu erhalten. In der Vorstudie erhielten weibliche Probanden während einer 28-tägigen Interventionsperiode täglich 2,4 g *t*11 und *t*12 (1:1, Testgruppe). In der Hauptstudie erhielt die Testgruppe (männliche und weibliche Probanden) täglich 6,0 g *t*11/*t*12 über 42 Tage. Weitere Probanden (Kontrollgruppe) erhielten ein *t*FS- und CLA-freies Kontroll-Öl. In der letzten Woche jeder Studienperiode bekamen die Probanden eine standardisierte Kost. In diesem Zeitraum wurde Urin und Stuhl gesammelt und Blut abgenommen

ERGEBNISSE

Fettsäurenanalyse Als Folge der *t*11 und *t*12 Aufnahme stieg der Gehalt von *t*11, *t*12 und *c*9,*t*11 CLA in den Lipiden des Serums sowie in den Membranen der Erythrozyten und peripheren mononukleären Zellen signifikant an. Der Anteil von *c*9,*t*12 18:2 blieb hingegen unverändert, woraus für *t*12 eine Konversionsrate von 0 % resultiert. Die Konversionsrate der täglich 1,2 g bzw. 3,0 g *t*11 lag gemessen im Serum bei 20 % bzw. 25 %. Außerdem erwiesen sich die Lipide der Erythrozytenmembran als geeignete Biomarker für die *t*11- und *t*12-Aufnahme sowie der endogenen CLA-Synthese. In der Testgruppe stieg der *c*9,*t*11 CLA Anteil signifikant an, während dieser in der Kontrollgruppe signifikant abfiel. Die Konversionsrate von *t*11 wurde anhand der Lipidinkorporation in die Erythrozytenmembran und die peripheren mononukleären Zellen mit 19 % bzw. 18 % errechnet. Der Einbau von *t*12 in die Zellmembranen war signifikant höher als von *t*11. Es wurden inter-individuelle und geschlechts-spezifische Unterschiede beobachtet.

Genexpression Die Regulation verschiedener Gene in isolierten Monozyten war geschlechts-abhängig. So wurde in den Monozyten der weiblichen Probanden die Expression der *cyclooxygenase 2 (COX2)* und der *fatty acid translocase* erhöht. In den männlichen Probanden stieg die Expression des *peroxisome proliferator-activated receptor (PPAR)* γ, *glucose transporter (GLUT4)*, und *peroxisomal bifunctional enzyme* während der *t*11 and *t*12 angereicherten Diät signifikant an. Dagegen wurde in den männlichen Kontroll-Probanden die *PPARγ, GLUT1* und *GLUT4* Expression signifikant reprimiert. In den Monozyten der weiblichen Kontroll-Probanden war die Expression der untersuchten Gene um ein Vielfaches erhöht.

Klinisch relevante Biomarker Im Urin der Testgruppe war 8-iso-$PGF_{2\alpha}$, ein Biomarker für oxidativen Stress (COX2-unabhängig), im Vergleich zur Kontrollgruppe und den Basiswerten signifikant gestiegen. Jedoch waren die Konzentrationen von 15-kd-$PGF_{2\alpha}$ (COX2-abhängige Lipidperoxidation) und 8-oxodG (oxidative DNA-Schäden) im Urin und die Konzentrationen von Tocopherolen und Retinol im Plasma unverändert. Die Serumlipide, Lipoproteine und Biomarker des Immunsystems und der Inflammation (Interleukine, TNFα, CRP, Prostacyclin, $sPLA_2$, ICAM-1, Leptin, Adiponectin) unterlagen ebenfalls keinen Veränderungen.

Die beobachteten Effekte können auf *t*11 und/oder *t*12 oder auf die endogen synthetisierte *c*9,*t*11 CLA zurückgeführt werden. Es ist ebenfalls möglich, dass die gebildete *c*9,*t*11 CLA die potentiellen Wirkungen von *t*11 bzw. *t*12 kompensierte.

SCHLUSSFOLGERUNG Nach derzeitiger Datenlage ist es zu empfehlen, die *t*FS-Aufnahme, besonders aus industriell verarbeiteten Lebensmitteln, so gering wie möglich zu gestalten. Die *trans*-Isomeren *t*9- und *t*10-18:1, welche besonders in technisch partiell gehärteten Fetten und Ölen vorliegen, sind keine Substrate für die Δ9-Desaturase. Im Gegensatz dazu, ist die *t*11, welche im Wiederkäuerfett das häufigste *trans* 18:1 Isomere ist, ein geeigneter Präkursor für die *c*9,*t*11 CLA-Synthese. Die Δ9-Desaturation der *t*11 ist durch den signifikanten Anstieg von *c*9,*t*11 CLA während der *t*11-Supplementation in den vorgestellten Studien evident. Die *t*12 hingegen wurde nicht zu *c*9,*t*12 18:2 desaturiert. Die Δ9-Desaturation ist gegenwärtig das Hauptkriterium, welches die *t*11 von anderen *trans* 18:1 Isomeren wie *t*9- und *t*10-18:1, und resultierend aus diesen Studien, der *t*12 unterscheidet. Die vorliegenden Studien zeigen, das *t*11 ein geeigneter Präkursor für die endogene *c*9,*t*11 CLA-Synthese ist und damit zur CLA-Versorgung des Menschen beiträgt.

APPENDIX

TABLE A-1

Distribution of total fatty acids in serum lipids after *t*11 and *t*12 supplementation (Σ 2.4 g/d) over 28 d of female control and test groups [% of Σ FAME].

Fatty acids	Adaptation period		Intervention period	
	Control group (n = 6)	Test group (n = 6)	Control group (n = 6)	Test group (n = 5)
	d 0	d 0	d 28	d 28
	Fatty acid distribution of serum lipids			
16:0	20.37 ± 1.65^{b}	20.75 ± 1.96^{b}	20.25 ± 1.28^{b}	22.67 ± 1.83^{a}
16:1	2.52±0.76	2.74 ± 0.50	2.36 ± 0.56	2.49 ± 0.72
18:0	8.37 ± 0.99	9.01 ± 0.73	8.55 ± 0.87	8.17 ± 0.48
***c*9 18:1**	16.92 ± 1.78	17.24 ± 0.83	17.23 ± 1.98	16.29 ± 1.50
***t*11 18:1**	0.11 ± 0.08^{b}	0.10 ± 0.02^{b}	0.10 ± 0.04^{b}	0.55 ± 0.29^{a}
***t*12 18:1**	0.10 ± 0.03^{b}	0.08 ± 0.01^{b}	0.09 ± 0.03^{b}	0.69 ± 0.22^{a}
***c*11 18:1**	2.47 ± 0.41	2.58 ± 0.19	2.49 ± 0.38	2.28 ± 0.23
***c*12 18:1**	0.08 ± 0.02^{b}	0.07 ± 0.01^{b}	0.07 ± 0.02^{b}	0.25 ± 0.04^{a}
***c*9,*c*12 18:2**	29.31 ± 3.37^{a}	29.23 ± 1.74^{a}	29.18 ± 2.32^{a}	27.01 ± 2.59^{b}
***c*9,*t*12 18:2**	0.04 ± 0.04	0.02 ± 0.03	0.02 ± 0.03	0.04 ± 0.03
***c*9,*t*11 CLA**	0.21 ± 0.03^{b}	0.22 ± 0.03^{b}	0.19 ± 0.07^{b}	0.36 ± 0.18^{a}
Σ CLA	0.29 ± 0.04^{b}	0.27 ± 0.04^{b}	0.25 ± 0.09^{b}	0.44 ± 0.26^{a}
20:4	7.40 ± 1.50	8.54 ± 1.34	7.04 ± 1.63	8.01 ± 1.36^{a}
Σ C_{18}	59.08 ± 6.22	58.63 ± 3.98	59.20 ± 5.95	57.13 ± 6.25

Mean ± SD. ^{ab}Mean values with different superscript letters were significantly different (one-way ANOVA, $P < 0.05$).

TABLE A-2

Distribution of CLA isomers in serum lipids after *t*11 and *t*12 supplementation (Σ 2.4 g/d) over 28 d of female control and test groups [% of Σ CLA].

CLA isomer	Adaptation period		Intervention period	
	Control group (n = 6) d 0	Test group (n = 6) d 0	Control group (n = 6) d 28	Test group (n = 5) d 28
Σ *t*,*t*	9.70 ± 1.02	9.56 ± 2.35	9.54 ± 4.03	9.93 ± 2.99
***t*10,*t*12**	1.28 ± 0.55	1.86 ± 0.89	2.37 ± 2.04	1.53 ± 1.62
***t*9,*t*11**	4.11 ± 0.61	4.52 ± 1.35	2.99 ± 0.88	2.41 ± 1.10
Σ *c*,*t*/*t*,*c*	87.37 ± 1.86	88.61 ± 2.73	88.66 ± 4.37	88.71 ± 2.43
***t*11,c13**	0.70 ± 0.37	0.83 ± 0.57	0.69 ± 0.31	3.10 ± 4.07
***c*11,*t*13**	1.15 ± 0.24	1.52 ± 0.45	1.32 ± 0.30	0.89 ± 0.63
***t*10,*c*12**	1.73 ± 1.10	2.22 ± 0.68	1.25 ± 0.36	1.12 ± 0.43
***c*9,*t*11**	75.24 ± 3.20^{ab}	76.49 ± 1.69^{ab}	75.98 ± 2.91^{b}	78.09 ± 2.54^{a}
***t*8,c10**	2.29 ± 0.80	2.79 ± 1.02	2.19 ± 0.79	2.24 ± 0.82
***t*7,c9**	5.51 ± 3.70	4.94 ± 1.32	5.47 ± 1.61	3.14 ± 0.89
Σ *c*,*c*	2.92 ± 0.99	1.86 ± 0.77	1.80 ± 0.97	1.88 ± 0.69
***c*10,*c*12**	0.33 ± 0.35	0.36 ± 0.40	0.21 ± 0.145	0.42 ± 0.25
***c*9,*c*11**	2.21 ± 0.90	1.49 ± 0.72	1.17 ± 0.69	1.24 ± 0.64

Mean ± SD. ^{ab}Mean values with different superscript letters were significantly different (one-way ANOVA, $P < 0.05$).

TABLE A-3

Distribution of CLA isomers in serum lipids after *t*11 and *t*12 supplementation (Σ 6.0 g/d) over 7 d and 42 d of control and test group [% of Σ CLA].

CLA isomer	Adaptation period		Intervention period		
	Control group (n = 12) d 0	Test group (n = 12) d 0	Test group (n = 11) d 7	Test group (n = 11) d 42	Control group (n = 12) d 42
Σ *t,t*	6.29 ± 3.05^{ab}	6.36 ± 2.64^{ab}	7.88 ± 3.53^{ab}	4.35 ± 3.67^{b}	7.01 ± 1.87^{a}
***t*10,*t*12**	1.15 ± 0.61	0.95 ± 0.54	1.29 ± 1.06	0.49 ± 0.34	1.45 ± 0.85
***t*9,*t*11**	3.06 ± 1.21	3.50 ± 2.47	2.96 ± 1.92	3.27 ± 3.05	4.38 ± 1.02
Σ *c,t/t,c*	90.96 ± 3.53^{ab}	90.76 ± 2.27^{ab}	89.46 ± 3.75^{ab}	93.05± 4.27^{b}	88.78 ± 2.64^{a}
***t*11,c13**	0.85 ± 0.43	1.25 ± 0.81	0.66 ± 0.13	0.23 ± 0.19	1.32 ± 1.12
***c*11,*t*13**	0.90 ± 0.43	0.70 ± 0.33	0.45 ± 0.23	0.52 ± 0.25	1.05 ± 0.56
***t*10,*c*12**	2.90 ± 2.15	2.71 ± 1.45	1.60 ± 0.82	2.30 ± 1.95	3.75 ± 2.16
***c*9,*t*11**	76.18 ± 4.43^{ab}	76.25 ± 4.17^{ab}	78.81 ± 5.21^{ab}	83.85 ± 4.77^{a}	72.08 ± 4.21^{b}
***t*8,c10**	2.83 ± 0.43	2.68 ± 0.48	2.96 ± 0.56	2.58 ± 0.92	3.29 ± 0.57
***t*7,c9**	5.52 ± 1.30	5.17 ± 1.26	4.29 ± 1.39	2.52 ±0.72	5.60 ± 1.18
Σ *c,c*	2.75 ± 1.21	2.90 ± 1.01	2.17 ± 1.14	2.60 ± 1.33	4.16 ± 1.53
***c*10,*c*12**	0.20 ± 0.26	0.36 ± 0.40	0.12 ± 0.05	0.15 ± 0.27	0.55 ± 0.84
***c*9,*c*11**	1.91 ± 0.47	2.06 ± 1.06	1.56 ± 0.82	2.19 ± 1.16	2.87 ± 0.9

Mean ± SD. ^{ab}Mean values with different superscript letters were significantly different (one-way ANOVA, $P < 0.05$).

TABLE A-4

Distribution of CLA isomers in lipids of red blood cell membranes after *t*11 and *t*12 supplementation (Σ 6.0 g/d) over 42 d of control and test group [% of Σ CLA].

CLA isomer	Adaptation period		Intervention period	
	Control group (n = 12) d 0	Test group (n = 12) d 0	Control group (n = 12) d 42	Test group (n = 11) d 42
Σ *t,t*	5.67 ± 3.35^{ab}	5.53 ± 2.47^{ab}	9.43 ± 3.88^{a}	4.64 ± 2.87^{b}
***t*10,*t*12**	0.85 ± 0.58	0.85 ± 0.54	1.39 ± 0.83	0.71 ± 1.43
***t*9,*t*11**	2.41 ± 1.42	2.96 ± 1.64	2.83 ± 1.84	1.80 ± 1.65
Σ *c,t/t,c*	89.31 ± 4.14^{ab}	89.29 ± 4.14^{ab}	86.06 ± 4.82^{a}	91.12 ± 6.84^{b}
***t*11,c13**	0.86 ± 1.33	0.76 ± 0.46	0.62 ± 0.34	1.13 ± 1.52
***c*11,*t*13**	1.49 ± 1.37	0.59 ± 0.67	1.21 ± 1.67	0.50 ± 1.75
***t*10,*c*12**	3.90 ± 2.81	2.34 ± 1.37	4.69 ± 2.37	2.21 ± 2.14
***c*9,*t*11**	78.04 ± 4.34^{ab}	78.73 ± 3.56^{ab}	75.01 ± 7.10^{a}	83.29 ± 7.30^{b}
***t*8,c10**	2.24 ± 0.99	2.04 ± 0.87	1.25 ± 0.50	1.36 ± 0.64
***t*7,c9**	2.31 ± 2.15	3.44 ± 2.02	2.05 ± 1.15	1.41 ±0.25
Σ *c,c*	4.98 ± 2.41	5.32 ± 3.67	4.61 ± 2.39	4.24 ± 0.45
***c*10,*c*12**	0.19 ± 0.30	0.11 ± 0.38	0.10 ± 0.24	0.05 ± 0.29
***c*9,*c*11**	4.36 ± 2.96	5.00 ± 3.88	4.51 ± 2.09	4.19 ± 0.45

Mean ± SD. ^{ab}Mean values with different superscript letters were significantly different (one-way ANOVA, $P < 0.05$).

TABLE A-5

Distribution of CLA isomers in lipids of feces after *t*11 and *t*12 supplementation (Σ 6.0 g/d) over 42 d of control and test group [% of Σ CLA].

CLA isomer	Adaptation period		Intervention period	
	Control group (*n* = 12) d 0	**Test group** (*n* = 12) d 0	**Control group** (*n* = 12) d 42	**Test group** (*n* = 11) d 42
Σ *t*,*t*	22.29 ± 8.99	24.71 ± 5.06	16.85 ± 5.72	17.71 ± 5.96
***t*10,*t*12**	2.54 ± 1.08	2.67 ± 1.22	2.56 ± 1.30	2.13 ± 1.22
***t*9,*t*11**	6.26 ± 2.43	8.42 ± 2.52	7.60 ± 2.57	8.07 ± 3.19
Σ *c*,*t*/*t*,*c*	74.67 ± 9.79	72.47 ± 5.38	79.84 ± 6.28	78.70 ± 6.77
***t*11,c13**	2.61 ± 2.07	3.10 ± 1.68	3.86 ± 2.39	2.40 ± 1.98
***c*11,*t*13**	1.16 ± 1.73	0.85 ± 0.93	0.92 ± 0.89	0.44 ± 0.57
***t*10,*c*12**	10.92 ± 11.10	6.20 ± 13.80	5.09 ± 4.68	4.12 ± 3.72
***c*9,*t*11**	57.28 ± 21.62	60.21 ± 18.77	66.79 ± 11.38	68.29 ± 10.65
***t*8,c10**	1. 51 ± 1.05	1.65 ± 1.15	3.18 ± 1.64	2.48 ± 1.11
***t*7,c9**	0.61 ± 0.46	0.55 ± 0.31	0.35 ± 0.20	0.39 ± 0.20
Σ *c*,*c*	2.61 ± 1.26	2. 28 ± 1.17	2.82 ± 1.17	3.12 ± 0.99
***c*10,*c*12**	0.26 ± 0.32	0.06 ± 0.36	0.16 ± 0.18	0.20 ± 0.26
***c*9,*c*11**	1.75 ± 0.85	1.45 ± 0.76	1.82 ± 0.75	2.12 ± 0.58

Mean ± SD. (one-way ANOVA, $P < 0.05$).

SELBSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Die Arbeit wurde bisher keiner anderen Prüfungsbehörde vorgelegt.

Jena, 6. Dezember 2006

Katrin Kuhnt

LIST OF PUBLICATIONS

Katrin Kuhnt, Jana Kraft, Peter Möckel, Gerhard Jahreis

Trans-11-18:1 is effectively Δ9-desaturated compared with *trans*-12-18:1 in humans.

***British Journal of Nutrition* 95, 752–61 (2006).**

Katrin Kuhnt, Andreas Wagner, Jana Kraft, Samar Basu, Gerhard Jahreis

Dietary supplementation with 11*trans*- and 12*trans*-18:1 and oxidative stress in humans.

***American Journal of Clinical Nutrition* 84, 981–8 (2006).**

Katrin Kuhnt, Jana Kraft, Heinz Vogelsang, Klaus Eder, Jürgen Kratzsch, Gerhard Jahreis

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American Journal of Clinical Nutrition, in preparation.

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41. Wissenschaftlicher Kongreß der Deutschen Gesellschaft für Ernährung, Freising-Weihenstephan, Germany; 11.-12. March 2004

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42. Wissenschaftlicher Kongress der Deutsche Gesellschaft für Ernährung, Kiel, Germany; 17.-18. March 2005

Humaninterventionsstudie zur Konversion von Vaccensäuren.

„Milchkonferenz“, Kiel, Germany; 29.-30. September 2005

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2. Jahrestagung der Deutschen Vereinten Gesellschaft für klinische Chemie und Laboratoriumsmedizin (DGKL), Jena, Germany; 6-8. October 2006

Erythrocyte membranes as biomarkers for fatty acid incorporation during a human intervention study.

43. Wissenschaftlicher Kongress der Deutsche Gesellschaft für Ernährung, Stuttgart/Hohenheim, Germany; 9.-10. March 2006

Humaninterventionsstudie zum Einfluss einer Supplementation von *trans*-Vaccensäure auf Biomarker des oxidativen Stresses.

Congress “Novel aspects of fatty acids (NAFA)”, Ystad, Schweden; 14-16. June 2006

Dietary supplementation with *t*11 and *t*12 C18:1 and oxidative stress in humans.

4th Euro fed Lipid Congress “Oils, fats, and Lipids for a healthier future”, Madrid, Spain, 29. September-4. October 2006

*t*11 and *t*12 C18:1 affects oxidative stress in humans.

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Katrin Kuhnt

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