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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 ($P \leq 0·002$). The serum *c*9,*t*11 CLA levels increased by 1·7- and 2·0-fold after 7 d and 42 d, respectively ($P \leq 0·001$). After 42 d, the test group's RBCM *c*9,*t*11 CLA content was elevated by 20 % ($P = 0·021$), whereas in the control group it was decreased by 50 % ($P = 0·002$). 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*trans* monoen in ruminant fats (30–80 % of total *trans*-18 : 1 isomers; Aro *et al.* 1998; Precht *et al.* 2001; unpublished data), depending on the cattle feeding regime (Jahreis *et al.* 1997; Bauman & Griinari, 2003; Kraft *et al.* 2003).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

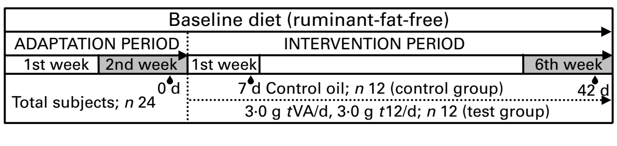

Fig. 1. Design of the intervention study. During an 8-week ruminant-fat-free baseline diet, twelve subjects (test group) received a *trans*-isomer mixture over 42 d, and twelve subjects received a control oil free of *trans*-fatty acids and CLA (control-group); ● blood sampling, ■ standardized diet over 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 $P < 0·05$ 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

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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 ($P \leq 0·005$; Table 3).

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 in the serum lipids of the test group after 7 d ($P=0·001$) and 42 d ($P=0·001$) 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) %; $P=0·004$). In contrast, after 42 d of intervention, the conversion rates of both genders were identical (men 23 (SD 6) %, women 26 (SD 11) %; $P=0·537$).

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 ($P=0·136$, $P=0·034$). In contrast, after 42 d, the *c*9,*t*11 CLA levels of the control group decreased to 72 (SD 4) % of total CLA ($P=0·010$) and were significantly lower than those found in the test group ($P=0·002$).

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 ($P = 0·002$), 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 ($P<0·05$).

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

≠ Not analysed after 7 d.

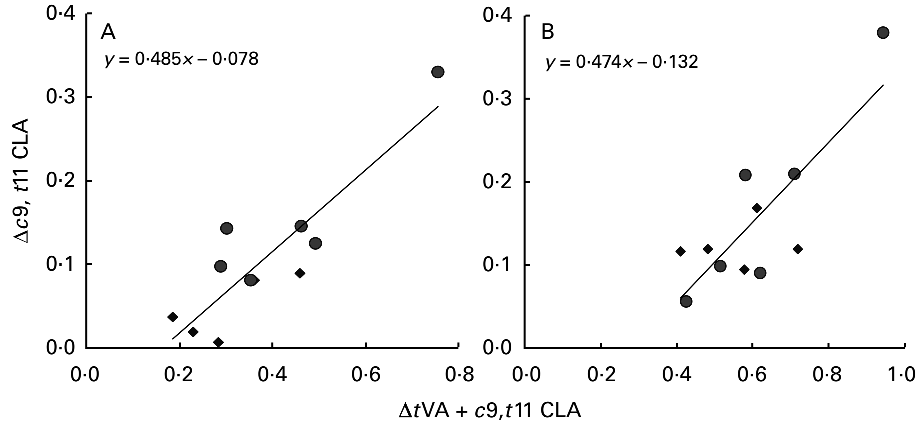

Fig. 2. Linear regression between the net change in *c*9,*t*11 conjugated linoleic acid (CLA (Δ*c*9,*t*11 CLA) and *trans*-vaccenic acid (Δ*t*VA) + Δ*c*9,*t*11 conjugated linoleic acid (CLA) in the serum lipids of the test group after 7 d (A) and 42 d (B) of intervention. The slope represents the average 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 ($P\leq0{\cdot}01$), 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)

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

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

	Salminen *et al.* 1998	Adlof *et al.* 2000	Turpeinen *et al.* 2002	Own results	
				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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