

## Effects of conjugated linoleic acid on linoleic and linolenic acid metabolism in man

Anu M. Turpeinen<sup>1\*</sup>, Sonja Bärlund<sup>1</sup>, Riitta Freese<sup>1</sup>, Peter Lawrence<sup>2</sup> and J. Thomas Brenna<sup>2</sup>

<sup>1</sup>Department of Applied Chemistry and Microbiology (Nutrition), University of Helsinki, PO Box 66, 00014 University of Helsinki, Finland

<sup>2</sup>Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA

(Received 16 September 2004 – Revised 11 January 2005 – Accepted 13 January 2005)

Evidence from animal studies suggests that conjugated linoleic acid (CLA) modulates plasma and tissue appearance of newly synthesized PUFA. The effects of a 1.2 g (0.5 % energy) daily intake of the *cis*-9,*trans*-11 (*c9,t11*) isomer of CLA, *trans*-10,*cis*-12 (*t10,c12*) isomer of CLA or olive oil (placebo) on linoleic acid (LA) and linolenic acid (LNA) metabolism in healthy human volunteers was investigated. Fifteen subjects were fed an experimental diet and supplemented with *c9,t11*-CLA, *t10,c12*-CLA or placebo for 7 d before consuming a tracer dose of U-<sup>13</sup>C]LA (50 mg) and U-<sup>13</sup>C]LNA (50 mg). Blood samples were taken at 0, 2, 4, 6, 8, 24, 48, 72 and 168 h and analysed using high-precision MS. No differences between the groups in peak plasma [<sup>13</sup>C]LA (10.3–11.6 % of dose), [<sup>13</sup>C]LNA (2.5–2.9 % of dose), [<sup>13</sup>C]arachidonic acid (0.09–0.12 % of dose), [<sup>13</sup>C]EPA (0.04–0.06 % of dose) or [<sup>13</sup>C]DHA (0.06–0.10 % of dose) were detected. Concentration *v.* time curves (area under the curve) also showed no significant differences between groups. This suggests that, in healthy human subjects consuming a diet with adequate intake of essential fatty acids, CLA does not affect metabolism of LA or LNA.

### Conjugated linoleic acid: Linoleic acid: Linolenic acid: Stable isotope: Man

A multitude of physiological effects for conjugated positional and geometrical isomers of linoleic acid (conjugated linoleic acid; CLA), specifically the *cis*-9,*trans*-11 (*c9,t11*) and *trans*-10,*cis*-12 (*t10,c12*) isomers, have been observed (Pariza *et al.* 2001), but the mechanisms involved are still unresolved. Altered eicosanoid synthesis, involving changes in the metabolism of essential fatty acids, has been proposed as a mechanism by which CLA may exert some of its effects (Banni *et al.* 1999*a,b*; Belury, 2002). Considering the multitude of functions for essential fatty acids in the body, this may result in widespread physiological effects.

CLA feeding is associated with altered tissue fatty acid composition, reflecting competition with other fatty acids for incorporation into tissues. Changes in saturated, monounsaturated and polyunsaturated fatty acids have been reported in animals (Belury & Kempa-Steczko, 1997; Banni *et al.* 1999*b*; Badinga *et al.* 2003; Ostrowska *et al.* 2003). Evidence from animal and cell culture studies suggests that CLA inhibits several enzymes involved in fatty acid metabolism, namely  $\Delta^9$ -desaturase,  $\Delta^6$ -desaturase and elongases (Bretillon *et al.* 1999; Chuang *et al.* 2001*a,b*; Sebedio *et al.* 2001; Loor *et al.* 2003).

Significant changes have been seen especially in linoleic acid (LA) and its metabolites. Tissue concentrations of LA and arachidonic acid (AA) decreased by up to 50 % in various animal species by feeding 0.5–1.5 % CLA (Belury &

Kempa-Steczko, 1997; Kramer *et al.* 1998; Banni *et al.* 1999*b*; Du *et al.* 2000; Ramsay *et al.* 2001). Effects have been most prominent in tissues containing neutral lipids, such as mammary and adipose tissue. Cell culture studies with pure isomers have shown that the *c9,t11* isomer of CLA is a stronger inhibitor of  $\Delta^6$ -desaturase and LA desaturation than the *t10,c12* isomer (Bretillon *et al.* 1999; Chuang *et al.* 2001*a*). Both *c9,t11*-CLA and *t10,c12*-CLA also inhibited elongation of LA by 20–60 % (Chuang *et al.* 2001*b*). Desaturation and elongation of  $\alpha$ -linolenic acid (LNA) was affected only slightly by both isomers (Bretillon *et al.* 1999).

Data in man are scarce. In supplementation studies, only minor changes in plasma fatty acids have been observed (Benito *et al.* 2001*a,b*; Mougios *et al.* 2001; Noone *et al.* 2002; Petridou *et al.* 2003). The only tracer study thus far found no effects of consuming 3.9 g CLA mixture on incorporation of deuterated oleic acid or LA in plasma lipid fractions (Emken *et al.* 2002). However, the concentration of desaturation and elongation products was too low to allow accurate quantification and it remains to be resolved whether LA metabolism is affected by CLA in man.

Our aim was to study the effects of two main CLA isomers, *c9,t11* and *t10,c12*, on the desaturation of LA and LNA in healthy human subjects using uniformly <sup>13</sup>C-labelled LA and LNA.

**Abbreviations:** AA, arachidonic acid; AUC, area under the curve; C, *cis*; CE, cholesterol esters; CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; GCC-IRMS, GC-combustion isotope ratio mass spectrometer; LA, linoleic acid; LNA, linolenic acid; PC, phosphatidylcholine; PE, phosphatidyl ethanolamine; *t*, *trans*; TAG, triacylglycerols.

\* **Corresponding author:** Dr Anu Turpeinen, fax +358 9 19158269, email anu.turpeinen@helsinki.fi

## Materials and methods

### Subjects

Fifteen healthy subjects participated in the study. Only healthy adults (>17 years), both male and female, were accepted. Pregnant or lactating women were excluded, as were subjects with any metabolic conditions. Subjects underwent health screening, consisting of a general health questionnaire and measurement of weight, height and blood pressure. A blood sample was analysed for lipid parameters and a urine sample for protein and glucose. Baseline characteristics of the subjects are presented in Table 1.

### Ethical considerations

The proposal was reviewed by the Ethical Committee of the University of Helsinki and by the Cornell University Committee on Human Subjects. The subjects gave their informed consent before entering the study.

### Experimental supplements and diet

Subjects were randomly allocated into three groups (four women and one man in each group) to receive *c9,t11* isomer of CLA, *t10,c12* isomer of CLA or olive oil as placebo (Natural Inc., Hovdebygda, Norway). Two gelatine-coated capsules, each containing 750 mg fatty acids, were consumed daily with lunch. The *c9,t11*-CLA capsules contained 79% of the *c9,t11* isomer, 7.8% of the *t10,c12* isomer, 5.8% of oleic acid (18:1), 0.3% of the *c9,c11* and *c10,c12* isomers, and 2.1% of the *t9,t11* and *t10,t12* isomers. The *t10,c12*-CLA capsules contained 84% of the *t10,c12* isomer, 10.6% of the *c9,t11* isomer, 1.6% of *c9-18:1*, 0.8% of the *c9,c11* and *c10,c12* isomers, and 2.3% of the *t9,t11* and *t10,t12* isomers. The capsules thus provided 1.19 g *c9,t11*-CLA/d and 1.26 g *t10,c12*-CLA/d. Olive oil capsules contained 73.5% 18:1 and <1% CLA. Compliance assessed by capsule counting was 99%. The subjects and personnel who analysed plasma samples were blinded to the treatment.

The total length of the study was 14 d. Subjects were free-living, but their fat intake was controlled. On weekdays, the subjects came to have a standardized lunch at the University

premises. On Fridays, ready-to-eat meals were provided for Saturday and Sunday. The subjects were also supplied with olive oil, margarine, bread and bakery products containing olive oil and instructed on how much to consume each day. They were also given a list of foods and food items prohibited during the study (fish and foods containing CLA, e.g. fatty milk products, beef, lamb and turkey). All subjects kept a 3 d food record, consisting of two weekdays and one day during the weekend. Energy and nutrient intakes were calculated using the Flamingo nutrition calculation program (version 1.3.2, 2000; Dipper Software, Helsinki, Finland). The calculated composition of the diets is shown in Table 2.

### Dosing

After 6 d on the experimental diet, on the morning of day 7, the subjects gave a fasting blood sample before ingesting approximately 50 mg U-<sup>13</sup>C]LA (46.0–51.4 mg) and 50 mg U-<sup>13</sup>C]LNA (48.4–52.6 mg) with a standard breakfast. The tracer dose was weighed individually for each subject and injected into a sugar cube. The breakfast consisted of a muffin (containing 10 g olive oil), orange juice, fat-free yoghurt and coffee or tea. Uniformly <sup>13</sup>C-labelled LA (free fatty acid, 98% purity) was purchased from Spectra Stable Isotopes (Columbia, MD, USA). Uniformly <sup>13</sup>C-labelled LNA (ethyl ester, 98% purity) was purchased from Cambridge Isotope Labs (Andover, MA, USA).

### Sampling

Blood samples were taken at 2, 4, 6 and 8 h after the dose. After the 4 h blood sample, the subjects had lunch and after the 6 h sample they were provided with an orange as a snack. Thereafter the subjects continued on the diet and overnight fasting blood samples were taken on three consecutive mornings. The last sample was taken 1 week (168 h) after the dosing.

Blood was taken into Vacutainer™ EDTA tubes and kept on ice before separating plasma by centrifugation. Plasma was snap-frozen and stored at –70°C.

### Analyses

Plasma total fatty acids were analysed from all samples. Total lipids were extracted using a modified Bligh and Dyer method

**Table 1.** Baseline characteristics of subjects (Mean values with their standard deviations)

	Study group						P*
	<i>c9,t11</i> -CLA		<i>t10,c12</i> -CLA		Control		
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	36.4	14.4	26.0	6.9	32.0	14.8	0.44
BMI (kg/m <sup>2</sup> )	23.4	4.2	23.0	2.5	22.7	2.3	0.93
Systolic blood pressure (mmHg)	123	19	122	14	114	10	0.56
Diastolic blood pressure (mmHg)	78	12	79	9	73	10	0.63
Serum cholesterol (mmol/l)	4.8	0.6	5.0	0.9	4.3	0.3	0.92
Serum triacylglycerols (mmol/l)	1.6	1.3	1.8	1.1	0.9	0.1	0.64
Serum HDL-cholesterol (mmol/l)	2.2	0.5	2.0	0.3	2.1	0.5	0.70
Serum LDL-cholesterol (mmol/l)	2.9	0.5	3.1	0.9	2.9	1.0	0.89

*c9,t11*-CLA, *cis*-9,*trans*-11 conjugated linoleic acid; *t10,c12*-CLA, *trans*-10,*cis*-12 conjugated linoleic acid.

\* Difference between groups (one-way ANOVA).

For details of subjects and procedures, see this page.

**Table 2.** Calculated composition of diets based on food records  
(Mean values with their standard deviations)

	Study group						<i>P</i> *
	c9,t11-CLA		t10,c12-CLA		Control		
	Mean	SD	Mean	SD	Mean	SD	
Energy (MJ)	9.8	2.6	9.0	1.1	9.3	2.2	0.85
Protein (% energy)	11.3	2.3	13.4	1.7	10.7	2.6	0.18
Carbohydrates (% energy)	47.5	7.6	45.4	3.2	50.1	7.2	0.52
Fat (% energy)	37.5	5.9	37.4	4.6	35.3	4.5	0.75
SFA (% energy)	6.7	1.3	7.2	2.3	6.1	1.7	0.60
MUFA (% energy)	19.7	3.9	18.4	3.4	18.0	2.0	0.76
PUFA (% energy)	5.9	1.0	4.9	0.8	4.8	1.0	0.17
LA (% energy)	5.1	0.8	4.2	6.3	4.3	1.1	0.32
LNA (% energy)	0.9	0.1	0.7	0.3	0.6	0.3	0.21
Cholesterol (mg)	110	65	106	16	97	40	0.90

c9,t11-CLA, *cis*-9,*trans*-11 conjugated linoleic acid; t10,c12-CLA, *trans*-10,*cis*-12 conjugated linoleic acid; SFA, saturated fatty acids; LA, linoleic acid; LNA, linolenic acid.

\*Difference between groups (one-way ANOVA).

For details of diets and procedures, see p. 728.

(Bligh & Dyer, 1959; Scheaff *et al.* 1995) and derivatized to fatty acid methyl esters (FAME) with BF<sub>3</sub> in methanol. FAME were dissolved in heptane with butylated hydroxytoluene added to prevent oxidation. Freshly prepared triheptadecanoic acid (Matreya Inc., Pleasant Gap, PA, USA) was added as internal standard to each sample. The total fatty acids composition was determined by GC (HP 5890 GC with flame ionization detector; Hewlett Packard, Palo Alto, CA, USA), using a CPSil 88 for FAME capillary column (100 m × 0.25 mm internal diameter, 0.2 μm film thickness; Chrompak, Middelburg, The Netherlands). H<sub>2</sub> was used as carrier gas with a flow rate of 1.7 ml/min. The temperature program used was from 80°C to 170°C at 10°C/min, then to 195°C at 1°C/min and finally to 225°C at 10°C/min, where it was held for 30 min. Total run time was 67 min. Response factors for each fatty acid were obtained by an equal weight FAME mixture and calibrated with methyl heptadecanoate (Matreya Inc.) as an internal standard.

Main plasma lipid fractions were separated by TLC from 8 h samples. Triacylglycerols (TAG) and cholesterol esters (CE) were separated on a Silica G plate (Analtech, Newark, DE, USA) with hexane–diethyl ether–88% formic acid (80:20:2, by vol.). Phosphatidyl ethanolamine (PE) and phosphatidylcholine (PC) were separated on a Silica H plate (Analtech) using a solvent mixture of chloroform–methanol–acetic acid–water (50:25:7:3, by vol.). TAG, CE, PC and PE standards (Matreya Inc. and Sigma, St. Louis, MO, USA) were used to identify the respective bands.

Tracer analyses for <sup>13</sup>C were performed using a high-precision GC–combustion isotope ratio mass spectrometer (GCC-IRMS; Finnigan MAT 252, Bremen, Germany) described in detail previously (Goodman & Brenna, 1992).

### Calculations

The concentration of tracer in tissues was calculated from the concentration of each fatty acid detected by GC and the atom percent excess (APE) of each fatty acid determined by the GCC-IRMS. Briefly, high-precision data are presented as the relative deviation from the international standard Pee Dee

Belemnite (PDB), with a <sup>13</sup>C:<sup>12</sup>C isotope ratio (*R*<sub>PDB</sub>) of 0.0112372, as:

$$\delta^{13}\text{C}_{\text{PDB}} = \frac{R_f - R_{\text{PDB}}}{R_{\text{PDB}}} \times 1000, \quad (1)$$

where *R<sub>f</sub>* is the ratio of the heavy to light isotope for the sample. Extracting *R<sub>f</sub>* from equation (1), atom percent (AP) can be calculated, which is the percentage of the heavier isotope in the analyte peak:

$$\text{AP}_f = \frac{R_f}{1 + R_f} \times 100. \quad (2)$$

APE is calculated by subtracting the baseline sample (0 h sample) from AP<sub>f</sub>. The total amount of tracer can be calculated by multiplying the APE by the tracee concentration (*Q<sub>f</sub>*) in tissues. This value is termed the molar dose equivalent:

$$D^* = \frac{\text{APE}_f}{100} \times Q_f \quad (3)$$

To eliminate artifactual differences resulting from different oral amounts, results are normalized by the amount of labelled fatty acid consumed by the subject (*d*):

$$\% \text{Dose} = \frac{D^*}{d}. \quad (4)$$

The % dose shows how much of the labelled fatty acid was transformed into products in terms of molar equivalents of the dosed fatty acids, [<sup>13</sup>C]LA into [<sup>13</sup>C]AA and [<sup>13</sup>C]LNA into [<sup>13</sup>C]EPA and [<sup>13</sup>C]DHA.

Concentration of the labelled fatty acid was plotted *v.* time (h) for each fatty acid and the total area under the curve (AUC; ng/ml plasma) was calculated according to Matthews *et al.* (1990) to obtain a relative measure of the amount of label appearing in a given fraction over the specified time period (168 h).

### Statistical analyses

Results are expressed as mean and standard deviation. Differences between groups in baseline parameters, dietary data, fatty acids, AUC data and tracer data were analysed using

one-way ANOVA with the Tukey HSD *post hoc* test (SPSS 10.0; SPSS Inc., Chicago, IL, USA). Significance was declared at  $P < 0.05$ .

## Results

All subjects successfully completed the study. The controls were taller than subjects in the CLA groups, but there were no other differences in baseline characteristics (Table 1). There were also no differences in dietary intake, based on 3 d food records, between the three groups (Table 2). Percentage of energy intake from fat was on average 37, from protein 12 and from carbohydrates 48. LA intakes in the *c9,t11*-CLA, *t10,c12*-CLA and control groups were 5.1%, 4.2% and 4.3% energy, respectively, and LNA intakes 0.9%, 0.7% and 0.6% energy, respectively.

### Plasma total and lipid fraction fatty acids

Plasma total fatty acids showed expected differences between the groups in the concentrations of *c9,t11*-CLA and *t10,c12*-CLA (Table 3). DHA was higher in both CLA groups compared with controls ( $P = 0.02$ ). Other major fatty acids did not reveal significant differences. Also, saturated:MUFA ratios, 16:0:16:1 and 18:0:18:1, did not differ between the groups.

There were significant differences in incorporation of the CLA isomers to specific plasma fractions. Highest concentrations of *c9,t11*-CLA were seen in TAG (0.5 wt%), whereas *t10,c12*-CLA was mainly incorporated into phospholipids, especially PC (0.2 wt%). *t10,c12*-CLA was not detected in all fractions in all samples.

Changes in other major fatty acids in plasma lipid fractions were few (Table 4). Concentrations of palmitic acid (16:0) in CE were higher in both the *c9,t11*-CLA and *t10,c12*-CLA groups than in controls ( $P = 0.047$ ). In TAG, 18:1 was lower

in the *c9,t11*-CLA group than in the *t10,c12*-CLA and control groups ( $P = 0.037$ ). However, no differences were seen in the ratios 16:0:16:1 and 18:0:18:1.

### Tracer analyses

[<sup>13</sup>C]LA and [<sup>13</sup>C]LNA increased rapidly in plasma after tracer intake. [<sup>13</sup>C]LNA reached its peak concentration at 2 h and had almost returned to baseline at 72 h. The peak for [<sup>13</sup>C]LA was at 8 h and its plasma concentration had almost returned to baseline at the end of the study (168 h). The mean amount of [<sup>13</sup>C]AA, [<sup>13</sup>C]EPA and [<sup>13</sup>C]DHA in plasma increased slowly over time and peak concentrations were reached at 48–72 h, 24–48 h and 48 h, respectively. Peak concentrations of AA were reached earlier in the *t10,c12*-CLA group than in the two other groups (24 h *v.* 48 h), but differences in plasma concentrations were not significant. At 168 h, [<sup>13</sup>C]EPA concentrations were near baseline whereas [<sup>13</sup>C]DHA concentrations were still considerably above baseline for most subjects.

Peak plasma concentrations of [<sup>13</sup>C]LA in the three groups were 4.7–5.6 ng/ml, which corresponds to 10.3–11.6% of the oral dose. The corresponding values were 1.0–1.3 ng/ml (2.5–2.9% of dose) for [<sup>13</sup>C]LNA, 0.04–0.06 ng/ml (0.09–0.12% of LA dose) for [<sup>13</sup>C]AA, 0.02–0.03 ng/ml (0.04–0.06% of LNA dose) for [<sup>13</sup>C]EPA and 0.06–0.09 ng/ml (0.06–0.10% of LNA dose) for [<sup>13</sup>C]DHA. No significant differences between the groups in peak plasma concentrations or AUC data up to 168 h were detected for any of the fatty acids (Table 5).

## Discussion

Evidence from animal and cell culture studies suggests that CLA affects tissue fatty acid composition, especially concentrations of LA and AA, by inhibiting  $\Delta^6$ -desaturase and elongase enzymes (Bretillon *et al.* 1999; Chuang *et al.* 2001a,b). We did not observe significant differences in desaturation or elongation of LA and LNA in healthy subjects consuming approximately 1.2 g (0.5% energy) *c9,t11*-CLA, *t10,c12*-CLA or olive oil daily, either in concentrations of labelled metabolites or plasma kinetics of tracer fatty acids or metabolites. We have no reason to believe that this was due to too short a CLA supplementation period (6 d) since in a previous study we showed that a steady state in plasma CLA is achieved in 4–6 d (Turpeinen *et al.* 2002). Also, the CLA dose used should have been sufficient to elicit any effects even at high dietary intake levels. The average daily intake has been estimated to be on average a few hundred milligrams (Ritzenthaler *et al.* 2001; Fremann *et al.* 2002). Finnish women are reported to have a habitual CLA intake of 130 mg/d (Aro *et al.* 2000).

A recent stable isotope study investigated the accretion and metabolism of deuterated oleic acid (*c9*–18:1-*d*<sub>8</sub>), LA (*c9,c12*–18:2-*d*<sub>2</sub>), *c9,t11*-CLA (*c9,t11*–18:2-*d*<sub>6</sub>) and *t10,c12*-CLA (*t10,c12*–18:2-*d*<sub>4</sub>) in six women who had consumed sunflower oil 6 g/d or CLA (mixture of isomers) 3.9 g/d for 63 d (Emken *et al.* 2002). In line with the present results, CLA isomers did not affect the incorporation of LA into plasma lipid fractions. However, only trace amounts of metabolites of LA were detected and thus the effects of CLA on desaturation and elongation of LA were not discussed. Since all other

**Table 3.** Total plasma fatty acids (wt%) of subjects fed *cis*-9,*trans*-11 conjugated linoleic acid (*c9,t11*-CLA), *trans*-10,*cis*-12 conjugated linoleic acid (*t10,c12*-CLA) or placebo

(Mean values with their standard deviations)

	Study group						<i>P</i> *
	<i>c9,t11</i> -CLA		<i>t10,c12</i> -CLA		Control		
	Mean	SD	Mean	SD	Mean	SD	
14:0	1.0	0.7	1.0	0.3	1.0	0.4	0.98
14:1	0.3	0.1	0.2	0.04	0.2	0.1	0.37
16:0	29.1	1.8	29.5	3.2	29.6	4.8	0.97
16:1	2.4	0.9	2.1	0.6	2.7	1.2	0.59
16:0:16:1	13.0	2.2	14.5	3.0	12.7	1.6	0.67
18:0:18:1	0.4	0.02	0.4	0.1	0.3	0.1	0.06
18:0	9.9	0.4	10.2	1.3	8.9	1.8	0.30
18:1	26.3	0.9	26.6	3.6	28.9	2.6	0.25
18:2 <i>n</i> -6	26.1	3.9	24.6	2.1	23.7	3.6	0.52
<i>c9,t11</i> -CLA	0.8	0.2	0.2	0.1	0.2	0.1	<0.001
<i>t10,c12</i> -CLA	0.04	0.05	0.5	0.2	<0.01		<0.001
18:3 <i>n</i> -3	0.7	0.2	0.7	0.1	0.7	0.2	0.76
20:3 <i>n</i> -6	0.2	0.1	0.2	0.1	0.6	0.8	0.34
20:4 <i>n</i> -6	1.1	0.1	1.3	0.2	1.4	0.2	0.06
20:5 <i>n</i> -3	0.1	0.1	0.2	0.1	0.1	0.1	0.36
22:6 <i>n</i> -3	1.0	0.3	0.9	0.2	0.5	0.2	0.02

\* Difference between groups (one-way ANOVA). For details of subjects and procedures, see p. 728.

**Table 4.** Fatty acids (wt%) in serum cholesterol esters (CE) and triacylglycerols (TAG) of subjects fed *cis*-9,*trans*-11 conjugated linoleic acid (*c9,t11*-CLA), *trans*-10,*cis*-12 conjugated linoleic acid (*t10,c12*-CLA) or olive oil placebo (control)

(Mean values with their standard deviations)

	Study group						<i>P</i> *
	<i>c9,t11</i> -CLA		<i>t10,c12</i> -CLA		Control		
	Mean	SD	Mean	SD	Mean	SD	
<b>CE</b>							
16:0	23.4	2.6	21.3	2.0	17.7	2.4	0.05
16:1	4.1	0.3	3.5	0.5	3.3	0.6	0.72
16:0:16:1	5.7	0.6	6.1	1.0	4.9	0.8	0.09
18:0	2.0	1.5	2.5	2.5	1.5	0.3	0.66
18:1	28.9	2.8	28.1	2.8	28.3	1.8	0.41
18:0:18:1	0.1	0.0	0.1	0.0	0.1	0.0	0.52
18:2 <i>n</i> -6	34.6	2.2	37.3	2.0	35.8	2.4	0.28
18:3 <i>n</i> -3	0.8	0.2	0.5	0.1	0.9	0.2	<0.01
<i>c9,t11</i> -CLA	0.2	0.0	0.0	0.0	0.1	0.0	<0.01
<i>t10,c12</i> -CLA	0.04	0.01	0.07	0.01	0.04	0.01	0.08
20:3 <i>n</i> -6	0.5	0.5	1.3	0.7	0.5	0.5	0.06
20:4 <i>n</i> -6	1.8	0.3	2.0	0.3	2.1	0.2	0.60
20:5 <i>n</i> -3	0.5	0.1	0.5	0.1	0.4	0.1	0.54
22:6 <i>n</i> -3	0.5	0.1	0.8	0.2	0.4	0.1	0.37
<b>TAG</b>							
16:0	29.1	1.7	28.3	1.9	30.9	1.0	0.12
16:1	3.0	0.4	3.0	0.6	3.4	0.2	0.24
16:0:16:1	9.8	1.3	9.5	1.3	9.1	0.9	0.57
18:0	21.1	2.0	20.1	1.4	20.5	1.7	0.61
18:1	30.2	1.8	32.9	2.0	33.2	1.4	0.04
18:0:18:1	0.7	0.1	0.6	0.1	0.6	0.1	0.08
18:2 <i>n</i> -6	14.1	1.4	12.6	1.3	12.5	0.7	0.10
18:3 <i>n</i> -3	0.6	0.2	0.5	0.2	0.6	0.2	0.68
<i>c9,t11</i> -CLA	0.8	0.2	0.1	0.1	0.2	0.1	<0.001
<i>t10,c12</i> -CLA	0.1	0.0	0.2	0.0	0.0	0.0	<0.001
20:4 <i>n</i> -6	0.9	0.2	0.9	0.2	0.9	0.2	0.95
20:5 <i>n</i> -3	0.6	0.2	0.5	0.2	0.5	0.1	0.10
22:6 <i>n</i> -3	0.4	0.1	0.5	0.1	0.5	0.1	0.75

\* Difference between groups (one-way ANOVA).

For details of subjects and procedures, see p. 728.

**Table 5.** Peak plasma concentrations (ng/ml plasma) and area under the curve\* (AUC; ng/ml plasma) for <sup>13</sup>C-labelled linoleic acid (LA), linolenic acid (LNA), arachidonic acid (AA), EPA and DHA in subjects fed *cis*-9,*trans*-11 conjugated linoleic acid (*c9,t11*-CLA), *trans*-10,*cis*-12 conjugated linoleic acid (*t10,c12*-CLA) or olive oil placebo (control)

(Mean values with their standard deviations)

	Study group						<i>P</i> †
	<i>c9,t11</i> -CLA		<i>t10,c12</i> -CLA		Control		
	Mean	SD	Mean	SD	Mean	SD	
<b>Peak concentration (ng/ml)</b>							
[ <sup>13</sup> C]LA	4.7	1.3	5.6	1.6	5.0	1.2	0.83
[ <sup>13</sup> C]LNA	1.3	0.3	1.1	0.7	1.1	0.4	0.84
[ <sup>13</sup> C]AA	0.06	0.02	0.05	0.02	0.05	0.03	0.93
[ <sup>13</sup> C]EPA	0.02	0.01	0.04	0.02	0.03	0.01	0.18
[ <sup>13</sup> C]DHA	0.09	0.05	0.09	0.03	0.06	0.03	0.47
<b>AUC (ng/ml)</b>							
[ <sup>13</sup> C]LA	642	302	719	243	603	302	0.74
[ <sup>13</sup> C]LNA	45	19	50	19	68	42	0.42
[ <sup>13</sup> C]AA	10	13	8.2	4.3	11	14	0.90
[ <sup>13</sup> C]EPA	3.6	2.9	5.8	2.4	3.7	1.0	0.26
[ <sup>13</sup> C]DHA	10	3.2	11	2.4	8.4	3.2	0.40

\* Total areas obtained from kinetic curves up to 168 h.

† Difference between groups (one-way ANOVA).

For details of subjects and procedures, see p. 728.

$^2\text{H}$ -labelled metabolites were also present at low concentrations or at levels below accurate quantification, this was probably a methodological issue rather than an indication of inhibited desaturation and elongation.

Generally, supplementing human subjects with 0.7–3.9 g CLA mixture/d has resulted in no or only minor changes in plasma fatty acids (Benito *et al.* 2001a; Mougios *et al.* 2001; Petridou *et al.* 2003). A significant decrease in LNA was detected when feeding a 50:50 mixture of *c9,t11*-CLA and *t10,c12*-CLA and a decrease in EPA when the same isomers were given in a 80:20 ratio (Noone *et al.* 2002). In a metabolic ward study with seventeen female subjects, CLA was incorporated into platelets at the expense of LA, but without changes in AA (Benito *et al.* 2001b).

In animals, on the other hand, inhibition of both  $\Delta^6$ -desaturase and elongase has been observed. Significant decreases (up to 50%) in LA metabolites have been reported in mammary and adipose tissue of rats (Banni *et al.* 1999b), pigs (Kramer *et al.* 1998; Ramsay *et al.* 2001) and hens (Du *et al.* 2000). Decreases in LA metabolites were associated with the appearance of conjugated metabolites of CLA, CD18:3 and CD20:3 (Sebedio *et al.* 2001), which do not serve as substrates for eicosanoid synthesis but rather inhibit it (Nugteren & Christ-Hazelhof, 1987). Liver and plasma were not affected (Sebedio *et al.* 2001), suggesting that the inhibitory effect of CLA may be seen in tissues with a high content of neutral lipids and CLA.

In tissues such as mammary and adipose tissue, the concentration of LA is low and competition between LA and CLA relatively favours CLA. When LA intake is also low, as in animal studies with fat-free or butter-fat diets, the situation is further aggravated. Due to the unavailability of these tissues from the present study it is not possible to determine whether this would have been the case in our subjects. However, considering that the average intake of LA exceeds that of CLA by over fifty-fold in most Western populations, the inhibitory effect of CLA is probably overcome by the excess of LA. The differences observed in human and animal studies in the effects of CLA on essential fatty acid metabolism may thus be partly explained by the high CLA:LA used in animal studies, not applicable to human studies. Also, species differences in the effects of CLA on gene expression, e.g. PPAR involved in the regulation of desaturases and other lipid-metabolizing enzymes, should be taken into account when interpreting results.

In line with previous studies (Banni *et al.* 2001; Sebedio *et al.* 2003), total CLA concentration was highest in TAG. *c9,t11*-CLA was incorporated mainly into TAG while accumulation of *t10,c12*-CLA was greatest in PC. The lower concentrations of *t10,c12*-CLA noted in the present as well as most other studies have been speculated to be due to increased metabolism because the *t10,c12* isomer is more easily oxidized because its structure allows it to bypass rate-limiting steps of peroxisomal  $\beta$ -oxidation (Martin *et al.* 2000). However, Burdge *et al.* (2004) did not observe differences in incorporation of the *c9,t11* and *t10,c12* isomers after accounting for higher baseline concentrations of *c9,t11*-CLA.

The changes noticed in concentrations of 16:0 (increase in both CLA groups in CE) and 18:1 (decrease in TAG in the *c9,t11* group) could be indications of decreased  $\Delta^9$ -desaturase activity. As a result, the concentration of saturated fatty acids increases at the expense of monounsaturates. However, the ratios 16:0:16:1 and 18:0:18:1, seen as indicators of

$\Delta^9$ -desaturase activity, did not differ between groups in total fatty acids or in plasma lipid fractions. Also, Burdge *et al.* (2004) reported no changes in lipid fraction fatty acids in healthy men supplemented with one, two or four capsules containing approximately 600 mg *c9,t11*-CLA or *t10,c12*-CLA for 8 weeks. In cell culture and animal studies, *t10,c12*-CLA has been shown to alter  $\Delta^9$ -desaturase activity, i.e. increase 18:0 content at the expense of 18:1 (and thus increase 18:0:18:1) or alter the ratio of 16:1:16:0 (Li & Watkins, 1998; Breillon *et al.* 1999; Du *et al.* 2000).

The decreases in *n*-6 fatty acids have occasionally been balanced by an increase in the content of long-chain PUFA (22:5 and 22:6) when feeding *t10,c12*-CLA, an effect hypothesized to be due to stimulation of the peroxisomal fatty acid metabolism (Li & Watkins, 1998; Du *et al.* 2000). Significant differences were not observed between groups in conversion of [ $^{13}\text{C}$ ]LNA to [ $^{13}\text{C}$ ]EPA or [ $^{13}\text{C}$ ]DHA, although increased concentrations of DHA in total plasma fatty acids were detected in both CLA groups compared with controls also in the present study. Since differences in DHA were not seen in plasma lipid fractions, it may be a chance result.

In conclusion, the present results indicate that the plasma appearance of long-chain PUFA derived from LA and LNA was not affected by an approximately 1.2 g daily intake of *c9,t11*-CLA or *t10,c12*-CLA in subjects with intake of essential fatty acids within recommendations. We cannot exclude differences in tissues, but it is probable that, with present intake levels of LA, the excess of LA overcomes any effects of CLA. This also suggests that the availability of precursors for eicosanoid synthesis is not affected by CLA.

### Acknowledgements

We gratefully acknowledge the cooperation of the subjects. This study was supported by the Cultural Foundation of Finland, the Juho Vainio Foundation, Finland, the Academy of Finland and National Institutes of Health (grant GM49209). None of the authors had any conflict of interest.

### References

- Aro A, Männistö S, Salminen I, Ovaskainen M-L, Kataja V & Uusitupa M (2000) Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutr Cancer* **38**, 151–157.
- Badinga L, Selberg KT, Dinges AC, Corner CW & Miles RD (2003) Dietary conjugated linoleic acid alters hepatic lipid content and fatty acid composition in broiler chickens. *Poult Sci* **82**, 111–116.
- Banni S, Angioni E, Carta G, *et al.* (1999a) Influence of dietary conjugated linoleic acid on lipid metabolism in relation to its anticarcinogenic activity. In *Advances in Conjugated Linoleic Acid Research*, vol. 1, pp. 307–318 [MP Yurawecz, MM Mossoba, JKG Kramer, MW Pariza and GJ Nelson, editors]. Champaign, IL: AOCS Press.
- Banni S, Angioni E, Casu V, Melis MP, Carta G, Corongiu FP, Thompson H & Ip C (1999b) Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. *Carcinogenesis* **20**, 1019–1024.
- Banni S, Carta G, Angioni E, Murru E, Scanu P, Melis MP, Bauman DE, Fischer SM & Ip C (2001) Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver. *J Lipid Res* **42**, 1056–1061.
- Belury MA (2002) Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *J Nutr* **132**, 2995–2998.

- Belury MA & Kempa-Steczko A (1997) Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* **32**, 199–204.
- Benito P, Nelson GJ, Kelley DS, Bartolini G, Schmidt PC & Simon V (2001a) The effect of conjugated linoleic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids* **36**, 221–227.
- Benito P, Nelson GJ, Kelley DS, Bartolini G, Schmidt PC & Simon V (2001b) The effect of conjugated linoleic acid on plasma lipoproteins and tissue fatty acid composition in humans. *Lipids* **36**, 229–236.
- Bligh E & Dyer W (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Bretillon L, Chardigny JM, Gregoire S, Bordeaux O & Sebedio JL (1999) Effects of conjugated linoleic acid isomers on the hepatic microsomal desaturation activities *in vitro*. *Lipids* **34**, 965–969.
- Burdge GC, Lupoli B, Russell JJ, *et al.* (2004) Incorporation of *cis*-9,*trans*-11 or *trans*-10,*cis*-12 conjugated linoleic acid into plasma and cellular lipids in healthy men. *J Lipid Res* **45**, 736–741.
- Chuang L-T, Thurmond JM, Liu JW, Kirchner SJ, Mukerji P, Bray TM & Huang Y-S (2001a) Effect of conjugated linoleic acid (CLA) on fungal delta-6-desaturase activity in a transformed yeast system. *Lipids* **36**, 139–143.
- Chuang L-T, Leonard AE, Liu J-W, Mukerji P, Bray TM & Huang Y-S (2001b) Inhibitory effect of conjugated linoleic acid on linoleic acid elongation in transformed yeast with human elongase. *Lipids* **36**, 1099–1103.
- Du M, Ahn DU & Sell JL (2000) Effects of dietary conjugated linoleic acid and linoleic:linolenic acid ratio on polyunsaturated fatty acid status in laying hens. *Poult Sci* **79**, 1749–1756.
- Emken EA, Adlof RO, Duval S, Nelson G & Benito P (2002) Effect of dietary conjugated linoleic acid (CLA) on metabolism of isotope-labeled oleic, linoleic, and CLA isomers in women. *Lipids* **37**, 741–750.
- Fremann D, Linseisen J & Wolfram G (2002) Dietary conjugated linoleic acid (CLA) intake assessment and possible biomarkers of CLA intake in young women. *Public Health Nutr* **5**, 73–80.
- Goodman KJ & Brenna JT (1992) High sensitivity tracer detection using high-precision gas chromatography–combustion isotope ratio mass spectrometry and highly enriched (U-<sup>13</sup>C)-labeled precursors. *Anal Chem* **64**, 1088–1095.
- Kramer JK, Sehat N, Dugan ME, Mossoba MM, Yurawecz MP, Roach JA, Eulitz K, Aalhus JL, Schaefer AL & Ku Y (1998) Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography. *Lipids* **33**, 549–558.
- Li Y & Watkins BA (1998) Conjugated linoleic acids alter bone fatty acid composition and reduce *ex vivo* prostaglandin E<sub>2</sub> biosynthesis in rats fed *n*-6 or *n*-3 fatty acids. *Lipids* **33**, 417–425.
- Loor JJ, Lin X & Herbein JH (2003) Effects of dietary *cis* 9, *trans* 11–18:2, *trans* 10, *cis* 12–18:2, or vaccenic acid (*trans* 11–18:1) during lactation on body composition, tissue fatty acids profiles, and litter growth in mice. *Br J Nutr* **90**, 1039–1048.
- Martin JC, Gregoire S, Siess MH, Genty M, Chardigny JM, Berdeaux O, Juaneda P & Sebedio JL (2000) Effects of conjugated linoleic acid isomers on lipid metabolizing enzymes in male rats. *Lipids* **35**, 91–98.
- Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *BMJ* **300**, 230–235.
- Mougiou V, Matsakas A, Petridou A, Ring S, Sagredos A, Melissopoulou A, Tsigilis N & Nikolaidis M (2001) Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. *J Nutr Biochem* **12**, 585–594.
- Noone EJ, Roche HM, Nugent AP & Gibney MJ (2002) The effect of supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Br J Nutr* **88**, 243–251.
- Nugteren DH & Christ-Hazelhof E (1987) Naturally-occurring conjugated octadecatrienoic acids are strong inhibitors of prostaglandin biosynthesis. *Prostaglandins* **33**, 403–417.
- Ostrowska E, Cross RF, Muralitharan M, Bauman DE & Dunshea FR (2003) Dietary conjugated linoleic acid differentially alters fatty acid composition and increases conjugated linoleic acid content in porcine adipose tissue. *Br J Nutr* **90**, 915–928.
- Pariza MW, Park Y & Cook ME (2001) The biologically active isomers of conjugated linoleic acid. *Prog Lipid Res* **40**, 283–298.
- Petridou A, Mougiou V & Sagredos A (2003) Supplementation with CLA: isomer incorporation into serum lipids and effect on body fat of women. *Lipids* **38**, 805–811.
- Ramsay TG, Evock-Clover CM, Sttele NC & Azain MJ (2001) Dietary conjugated linoleic acid alters fatty acid composition in pig skeletal muscle and fat. *J Anim Sci* **79**, 2152–2161.
- Ritzenthaler KL, McGuire MK, Falen R, Shultz TD, Dasgupta N & McGuire MA (2001) Estimation of conjugated linoleic acid intake by written dietary assessment methodologies under estimates actual intake evaluated by food duplicate methodology. *J Nutr* **131**, 1548–1554.
- Scheaff RC, Su H-M, Keswick LA & Brenna JT (1995) Conversion of  $\alpha$ -linolenate to docosahexaenoate is not depressed by high dietary levels of linoleate in young rats: tracer evidence using high precision mass spectrometry. *J Lipid Res* **36**, 998–1008.
- Sebedio JL, Angioni E, Chardigny JM, Gregoire S, Juaneda P & Berdeaux O (2001) The effect of conjugated linoleic acid isomers on fatty acid profiles of liver and adipose tissues and their conversion to isomers of 16:2 and 18:3 conjugated fatty acids in rats. *Lipids* **36**, 575–582.
- Sebedio JL, Chardigny JM & Berdeaux O (2003) Metabolism of conjugated linoleic acids. In *Advances in Conjugated Linoleic Acid Research*, pp. vol. 2, 259–266 [JL Sebedio, WW Christie and R Adlof, editors]. Champaign, IL: AOCS Press.
- Turpeinen AM, Mutanen M, Aro A, Salminen I, Basu S, Palmquist DL & Griinari JM (2002) Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am J Clin Nutr* **76**, 504–510.