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

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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-[13C]LA (50 mg) and U-[13C]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 [13C]LA (10·3–11·6 % of dose), [13C]LNA (2·5–2·9 % of dose), [13C]arachidonic acid (0·09–0·12 % of dose), [13C]EPA (0·04–0·06 % of dose) or [13C]DHA (0·06–0·10 % of dose) were detected. Concentration vs. 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. 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. 1999a,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. 1999b; 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 Δ^9-desaturase, Δ^6-desaturase and elongases (Brețillon et al. 1999; Chuang et al. 2001a,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. 1999b; 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 Δ^9-desaturase and LA desaturation than the t10,c12 isomer (Brețillon et al. 1999; Chuang et al. 2001a). Both c9,t11-CLA and t10,c12-CLA also inhibited elongation of LA by 20–60 % (Chuang et al. 2001b). Desaturation and elongation of α-linolenic acid (LNA) was affected only slightly by both isomers (Brețillon et al. 1999).

Data in man are scarce. In supplementation studies, only minor changes in plasma fatty acids have been observed (Benito et al. 2001a,b; Mougiou 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 [13C]-labelled LA and LNA.
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-CLA, t10,c12 isomer of CLA or olive oil as placebo (Natuve Inc., Hovdebygd, 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 thus provided 1.19 g t9,c12 conjugated linoleic acid; and 2.3 g t10,c12 conjugated linoleic acid.

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-[13C]LNA (46.0–51.4 mg) and 50 mg U-[13C]LINA (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 13C-labelled LA (free fatty acid, 98% purity) was purchased from Spectra Stable Isotopes (Columbia, MD, USA). Uniformly 13C-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

<table>
<thead>
<tr>
<th>Study group</th>
<th>c9,t11-CLA</th>
<th>t10,c12-CLA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 4.2</td>
<td>23.0 ± 2.5</td>
<td>22.7 ± 2.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123 ± 19</td>
<td>122 ± 14</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 12</td>
<td>79 ± 9</td>
<td>73 ± 10</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>4.8 ± 0.6</td>
<td>5.0 ± 0.9</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Serum triacylglycerol (mmol/l)</td>
<td>1.6 ± 1.3</td>
<td>1.8 ± 1.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>2.2 ± 0.5</td>
<td>2.0 ± 0.3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>2.9 ± 0.5</td>
<td>3.1 ± 0.9</td>
<td>2.9 ± 1.0</td>
</tr>
</tbody>
</table>

* Difference between groups (one-way ANOVA).

For details of subjects and procedures, see this page.
(Bligh & Dyer, 1959; Scheaff et al. 1995) and derivatized to fatty acid methyl esters (FAME) with BF3 in methanol. FAME were dissolved in heptane with butylated hydroxytoluene added to prevent oxidation. Freshly prepared triheptadecanoin (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; Chrompak, Middelburg, The Netherlands). H2 was used as carrier gas with a 0·2 µm film thickness; Chrompak, Middelburg, The Netherlands). H2 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 (Anatech, 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, Newark, DE, USA) using a solvent mixture of chloroform–methanol–acetic acid (80:20:2, by vol.). Phosphatidyl ethanolamine (PC) and phosphatidylcholine were separated on a Silica G plate (Analtech, Newark, DE, USA) with hexane–diethyl ether–88% formic acid (80:20:2, by vol.).

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 13C:12C isotope ratio (R_PDB) of 0.0112372, as:

\[
\delta^{13}C_{\text{PDB}} = \frac{R_f - R_{\text{PDB}}}{R_{\text{PDB}}} \times 1000.
\]

where \( R_f \) is the ratio of the heavy to light isotope for the sample. Extracting \( R_f \) from equation (1), atom percent (AP) can be calculated, which is the percentage of the heavier isotope in the analyte peak:

\[
AP_f = \frac{R_f}{1 + R_f} \times 100.
\]

APE is calculated by subtracting the baseline sample (0 h sample) from \( AP_f \). The total amount of tracer can be calculated by multiplying the APE by the tracee concentration (\( Q_t \)) in tissues. This value is termed the molar dose equivalent:

\[
D^* = \frac{APE_f \times Q_t}{100}.
\]

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

\[
\% \text{Dose} = \frac{D^*}{d}.
\]

The % dose shows how much of the labelled fatty acid was transformed into products in terms of molar equivalents of the dosed fatty acids. [13C]LA into [13C]AA and [13C]LNA into [13C]EPA and [13C]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. The calculated composition of diets based on food records is shown in Table 2.

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

<table>
<thead>
<tr>
<th>Study group</th>
<th>c9,t11-CLA</th>
<th>t10,c12-CLA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>9·8</td>
<td>2·6</td>
<td>9·0</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>11·3</td>
<td>2·3</td>
<td>13·4</td>
</tr>
<tr>
<td>Carbohydrates (% energy)</td>
<td>47·5</td>
<td>7·6</td>
<td>45·4</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td>37·5</td>
<td>5·9</td>
<td>37·4</td>
</tr>
<tr>
<td>SFA (% energy)</td>
<td>6·7</td>
<td>1·3</td>
<td>7·2</td>
</tr>
<tr>
<td>MUFA (% energy)</td>
<td>19·7</td>
<td>3·9</td>
<td>18·4</td>
</tr>
<tr>
<td>PUFA (% energy)</td>
<td>5·9</td>
<td>1·0</td>
<td>4·9</td>
</tr>
<tr>
<td>LA (% energy)</td>
<td>5·1</td>
<td>0·8</td>
<td>4·2</td>
</tr>
<tr>
<td>LNA (% energy)</td>
<td>0·9</td>
<td>0·1</td>
<td>0·7</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>110</td>
<td>65</td>
<td>106</td>
</tr>
</tbody>
</table>

* Difference between groups (one-way ANOVA).
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 carbo-
hydrates 48%. LA intakes in the c9,t11-CLA, t10,c12-CLA and
group controls 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,
with controls (Mean values with their standard deviations)
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 concen-
trations 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

[13C]LA and [13C]LNA increased rapidly in plasma after
tracer intake. [13C]LNA reached its peak concentration at 2 h
and had almost returned to baseline at 72 h. The peak for
[13C]LA was at 8 h and its plasma concentration had almost
returned to baseline at the end of the study (168 h). The
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
r10,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, [13C]EPA concentrations were near baseline
whereas [13C]DHA concentrations were still considerably
above baseline for most subjects.

Peak plasma concentrations of [13C]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–
29% of dose) for [13C]LNA, 0.04–0.06 ng/ml (0.09–0.12 %
of LA dose) for [13C]AA, 0.02–0.03 ng/ml (0.04–0.06 %
of LNA dose) for [13C]EPA and 0.06–0.09 ng/ml (0.06–0.10 %
of LNA dose) for [13C]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 concen-
trations of LA and AA, by inhibiting Δ6-desaturase and elon-
gase 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 metab-
olites. 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
(Ritzenhaller 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-d9), LA
(c9,c12–18:2-d2), c9,t11-CLA (c9,c12–18:2-d2) and
t10,c12-CLA (t10,c12–18:2-d2) 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 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)

<table>
<thead>
<tr>
<th>Study group</th>
<th>c9,t11-CLA</th>
<th>t10,c12-CLA</th>
<th>Control</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>CE</td>
<td>16:0</td>
<td>23.4±2.6</td>
<td>21.3±2.0</td>
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<td>16:1</td>
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<td>4.9±0.8</td>
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<tr>
<td></td>
<td>18:0</td>
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<tr>
<td></td>
<td>18:1</td>
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<td>28.3±1.8</td>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>37.3±2.0</td>
<td>35.8±2.4</td>
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<td></td>
<td>18:3n-3</td>
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<tr>
<td></td>
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<td>0.5±0.1</td>
<td>0.8±0.2</td>
<td>0.4±0.1</td>
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<tr>
<td>TAG</td>
<td>16:0</td>
<td>29.1±1.7</td>
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<tr>
<td></td>
<td>18:2n-6</td>
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<tr>
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<td>0.4±0.1</td>
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</tr>
</tbody>
</table>

* 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 13C-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)

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

* 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.
$^{3}$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; Mougiou et al. 2001; Petridou et al. 2003). A significant decrease in LNA was detected when feeding a 50:50 mixture of $\text{c}_9\text{t}_11$-CLA and $\text{t}_10$,$\text{c}_12$-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 by 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. $\text{c}_9\text{t}_11$-CLA was incorporated mainly into TAG while accumulation of $\text{t}_10$,$\text{c}_12$-CLA was greatest in PC. The lower concentrations of $\text{t}_10$,$\text{c}_12$-CLA noted in the present as well as most other studies have been speculated to be due to increased metabolism because the $\text{t}_10$,$\text{c}_12$ 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 $\text{c}_9\text{t}_11$ and $\text{t}_10$,$\text{c}_12$ isomers after accounting for higher baseline concentrations of $\text{c}_9\text{t}_11$-CLA.

The changes noticed in concentrations of 16:0 (increase in both CLA groups in CE) and 18:1 (decrease in TAG in the $\text{c}_9\text{t}_11$ group) could be indications of decreased $\Delta^\text{9}$-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 $\Delta^\text{9}$-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 $\text{c}_9\text{t}_11$-CLA or $\text{t}_10$,$\text{c}_12$-CLA for 8 weeks. In cell culture and animal studies, $\text{t}_10$,$\text{c}_12$-CLA has been shown to alter $\Delta^\text{9}$-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; Bretillon 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 $\text{t}_10$,$\text{c}_12$-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 [13C]LNA to [13C]EPA or [13C]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 $\text{c}_9\text{t}_11$-CLA or $\text{t}_10$,$\text{c}_12$-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.

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References


