Opposing effects of \textit{cis}-9,\textit{trans}-11 and \textit{trans}-10,\textit{cis}-12 conjugated linoleic acid on blood lipids in healthy humans\textsuperscript{1,3}

Sabine Tricon, Graham C Burdge, Samantha Kew, Tapati Banerjee, Jennifer J Russell, Emma L Jones, Robert F Grimble, Christine M Williams, Parveen Yaqoob, and Philip C Calder

\textbf{ABSTRACT}

\textbf{Background:} Conjugated linoleic acid (CLA) is reported to have weight-reducing and antiatherogenic properties when fed to laboratory animals. However, the effects of CLA on human health and, in particular, the effects of individual CLA isomers are unclear.

\textbf{Objective:} This study investigated the effects of 3 doses of highly enriched \textit{cis}-9,\textit{trans}-11 (0.59, 1.19, and 2.38 g/d) or \textit{trans}-10,\textit{cis}-12 (0.63, 1.26, and 2.52 g/d) CLA preparations on body composition, blood lipid profile, and markers of insulin resistance in healthy men.

\textbf{Design:} Healthy men consumed 1, 2, and 4 capsules sequentially, containing either 80\% \textit{cis}-9,\textit{trans}-11 CLA or 80\% \textit{trans}-10,\textit{cis}-12 CLA for consecutive 8-wk periods. This phase was followed by a 6-wk washout and a crossover to the other isomer.

\textbf{Results:} Body composition was not significantly affected by either isomer of CLA. Mean plasma triacylglycerol concentration was higher during supplementation with \textit{trans}-10,\textit{cis}-12 CLA than during that with \textit{cis}-9,\textit{trans}-11 CLA, although there was no influence of dose. There were significant effects of both isomer and dose on plasma total cholesterol and LDL-cholesterol concentrations but not on HDL-cholesterol concentration. The ratios of LDL to HDL cholesterol and of total to HDL cholesterol were higher during supplementation with \textit{trans}-10,\textit{cis}-12 CLA than during that with \textit{cis}-9,\textit{trans}-11 CLA. CLA supplementation had no significant effect on plasma insulin concentration, homeostasis model for insulin resistance, or revised quantitative insulin sensitivity check index.


\textbf{KEY WORDS} Cholesterol, conjugated linoleic acid, high-density lipoprotein, low-density lipoprotein, triacylglycerol

\textbf{INTRODUCTION}

Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of linoleic acid, in which the 2 double bonds are conjugated. CLA is found in ruminant animal fat, dairy products, and partially hydrogenated vegetable oils. Several positive health effects were attributed to CLA, including anticarcinogenic effects (1–5), antiatherogenic effects (6, 7), and effects on body composition (reviewed in Roche et al; 8] and on blood lipid concentrations (6, 7, 9, 10). The effects of CLA on body composition and blood lipids in animal studies are relatively dramatic. However, they are not unequivocally supported by consistent data from human studies, because, in part, animal studies used much larger relative doses of CLA than human studies.

At least 5 human studies demonstrated no effect of CLA on body weight or composition (11–15), whereas 4 studies reported a reduction in fat mass as a result of CLA supplementation (16–19). The reported effects of CLA supplementation on blood lipids in humans are also contradictory. Three human studies showed that CLA supplementation at 3.9 g/d for 63 d, 4.2 g/d for 72 d, or 2.1 g/d for 45 d had no significant effect on plasma lipid concentrations (15, 18, 20). In contrast to those 3 studies, 3 g/d of a 50:50 isomeric blend of CLA (consisting of \textit{cis}-9,\textit{trans}-11 CLA and \textit{trans}-10,\textit{cis}-12 CLA) for 8 wk significantly decreased plasma triacylglycerol concentrations in normolipemic subjects, whereas an 80:20 mixture of \textit{cis}-9,\textit{trans}-11 CLA and \textit{trans}-10,\textit{cis}-12 CLA did not (13). A study using lower doses (0.7 g/d and 1.4 g/d) of a 50:50 mixture of these isomers demonstrated a tendency toward lowering plasma triacylglycerol and cholesterol concentrations and in addition reported a decrease in HDL-cholesterol concentration (17). However, overweight subjects supplemented with 1.7–6.8 g/d CLA preparation for 12 wk experienced significant reductions in total cholesterol, HDL-cholesterol, and LDL-cholesterol, but not in plasma triacylglycerol concentrations (16).

The \textit{cis}-9,\textit{trans}-11 CLA isomer is the principal dietary form of CLA, accounting for as much as 85–90\% of the total CLA content in dairy products (21). Interest is increasing in the biological effects of \textit{trans}-10,\textit{cis}-12 CLA, and emerging evidence suggests that the actions of the \textit{cis}-9,\textit{trans}-11 and \textit{trans}-10,\textit{cis}-12 isomers

\textsuperscript{1} From the Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading, United Kingdom (ST, SK, ELJ, CMW, and PY), and the Institute of Human Nutrition, University of Southampton, Southampton, United Kingdom (GCB, TB, JFR, RF, and PCC).

\textsuperscript{2} Supported by grant no. EFH/16 from the Biotechnology and Biological Sciences Research Council and by the Department of Environment, Food and Rural Affairs, the Scottish Executive Environmental and Rural Affairs Department, and the Milk Development Council, under the Eating, Food and Health LINK scheme (to PCC, PY, RF, and CMW). The capsules used in this study were a gift from Natural ASA (Hovedbygda, Norway).

\textsuperscript{3} Address reprint requests to P Yaqoob, Hugh Sinclair Unit of Nutrition, School of Food Biosciences, University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom. E-mail: p.yaqoob@reading.ac.uk.

Received October 22, 2003.

Accepted for publication March 12, 2004.
might be different (22). However, this evidence is indirect, because there are no human studies to date that have directly compared the effects of highly enriched preparations of each isomer. Most of the above-mentioned studies investigated the effects of mixtures of a large number of CLA isomers, one study compared an 80:20 and a 50:50 mix of cis-9,trans-11 CLA and trans-10,cis-12 CLA (13), and one study compared the 50:50 mix with pure trans-10,cis-12 CLA (23). The latter study, conducted in obese men with metabolic syndrome, demonstrated that trans-10,cis-12 CLA lowered HDL-cholesterol concentration and increased insulin resistance compared with the 50:50 mixture (23). This finding has raised concerns about the safety of supplementation with trans-10,cis-12 CLA.

It is clearly important to identify the separate effects of each isomer of CLA to clarify these safety issues. This study investigates, for the first time, the effects of highly enriched preparations of cis-9,trans-11 and trans-10,cis-12 CLA, each at 3 doses, on body composition, blood lipid concentrations, and markers of insulin resistance in healthy men.

SUBJECTS AND METHODS

Subjects and study design

The study was conducted at the University of Southampton and the University of Reading, with approval from the Research and Ethics Committee of the University of Reading and the South and West Hampshire Local Research Ethics Committee. Healthy male volunteers aged 20–47 y were recruited by advertisements, and potential volunteers were selected after confirming that they were healthy; had a body mass index (BMI) > 18 kg/m² and < 34 kg/m²; had no diagnosed cardiovascular disease, diabetes, liver or endocrine dysfunction, or chronic inflammatory disease; were not taking any medication; were not vegetarians or vegans; were not heavy smokers (≥10 cigarettes/d); were not heavy consumers of alcohol; and were not consuming any supplements (such as vitamins, fish oils, or evening primrose oil). Subjects fitting these criteria were then screened for eligibility. Subjects were weighed to the nearest kilogram. In the first arm of the study, body composition (percent of fat and lean mass) was measured at each visit by skinfold thickness at 4 sites and end of each 6-mo arm of the study, indicated no evidence of liver toxicity of either CLA isomer (data not shown). The body composition was measured by dual-energy X-ray absorptiometry (DXA). The second arm of the study only bioelectrical impedance analysis was conducted, and the results shown were obtained from these measurements.

Table 1

Characteristics of the treatment groups at the start of the crossover study.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>TAG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-9,trans-11 CLA</td>
<td>trans-10,cis-12 CLA</td>
<td>30.7 ± 1.7</td>
<td>77.6 ± 2.1</td>
</tr>
<tr>
<td>cis-9,trans-11 CLA</td>
<td>trans-10,cis-12 CLA</td>
<td>31.2 ± 1.7</td>
<td>77.9 ± 2.2</td>
</tr>
</tbody>
</table>

All values are μ ± SEM. CLA, conjugated linoleic acid; TAG, triacylglycerols. There were no significant differences between treatment groups (unpaired t-tests).

or trans-10,cis-12 CLA in triacylglycerol form. The cis-9,trans-11 CLA–enriched capsules contained 79.3% cis-9,trans-11 CLA, 7.8% trans-10,cis-12 CLA, 5.8% 18:1n–9, and 7.1% other fatty acids, whereas the trans-10,cis-12 CLA enriched capsules contained 84.1% trans-10,cis-12 CLA, 10.6% cis-9,trans-11 CLA, 1.7% 18:1n–9, and 5.3% other fatty acids. The fatty acid composition of the capsules was analyzed by gas chromatography, as described in Burdge et al. (24). The capsules used were identical in appearance and were packaged in the same manner. Subjects consumed 1, 2, or 4 capsules/d, which provided 0.59, 1.19, or 2.38 g/d cis-9,trans-11 CLA and 0.63, 1.26, or 2.52 g/d trans-10,cis-12 CLA, respectively. Mean compliance assessed by capsule counting was 92% and was not significantly different between doses and isomer treatment. The capsules were well tolerated. Analysis of liver enzymes, conducted at baseline and end of each 6-mo arm of the study, indicated no evidence of liver toxicity of either CLA isomer (data not shown).

Body weight and composition

Subjects were weighed to the nearest kilogram. In the first arm of the study, body composition (percent of fat and lean mass) was determined at each visit by skinfold thickness at 4 sites and application of the Siri equation (25), as well as by bioelectrical impedance analysis, using the Bodystat 1500 (Bodystat Limited, Douglas, United Kingdom). Pearson correlation analysis indicated a high degree of correlation between the 2 methods ($r = 0.725, P < 0.0001$ for percent of body fat). Therefore, in the second arm of the study only bioelectrical impedance analysis was conducted, and the results shown were obtained from these measurements.

Biochemical analysis

Blood samples were collected into heparinized evacuated tubes between 0800 and 1000 after a fast of at least 10 h. Plasma was prepared and stored at $-20$ °C before analysis. Plasma triacylglycerol, cholesterol, HDL-cholesterol, glucose (Instrumentation Laboratories Ltd, Warrington, United Kingdom) and non-esterified fatty acid (NEFA: Wako NEFA C kit; Alpha Laboratories Ltd, Eastleigh, United Kingdom) concentrations were measured with use of an ILab 600 clinical chemistry analyzer (Instrumentation Laboratories Ltd). Plasma insulin concentration was measured with use of a specific enzyme immunoassay (Dako Diagnosis Ltd, Cambridge, United Kingdom). LDL-cholesterol concentrations were determined by the Friedewald formula (26). Fasting insulin and glucose concentrations were used to calculate insulin resistance from the homeostasis model for insulin resistance (HOMA-IR) model ($[(\text{insulin}_0 \times \text{glucose}_0)/$
revised quantitative insulin sensitivity check index (QUICKI) formula \[1/(\log \text{glucose}_0 + \log \text{insulin}_0 + \log \text{NEFA}_0)\] (28). Statistical analysis

All statistical tests were performed with use of SPSS version 11.0 (SPSS Inc, Chicago), and a value of \( P < 0.05 \) was taken to indicate statistical significance. “Prestatistical” analyses were used to determine whether there were any period effects or treatment-period interaction as a result of the crossover design. This analysis was simplified by calculating the absolute change for each marker tested (mean value at baseline subtracted from the mean value at the highest dose of each isomer for each subject) and testing differences between periods with use of independent-sample \( t \) tests. There was no period effect and no treatment-period interaction (data not shown); thus, all data were treated as paired samples from a crossover study. Data that were not normally distributed were logarithmically transformed. Data were analyzed with use of two-factor repeated-measures analysis of variance (ANOVA) followed by post hoc analysis when relevant (one-factor repeated-measures ANOVA, followed by Tukey tests for a significant effect of dose, and paired \( t \) tests for a significant effect of isomer).

RESULTS

Effects of \( \text{cis-9,trans-11} \) conjugated linoleic acid and \( \text{trans-10,cis-12} \) conjugated linoleic acid on body weight, body mass index, and body composition

The effects of CLA on body weight, BMI, fat mass, and fat-free mass of subjects at baseline and after supplementation with each of the 3 doses of \( \text{cis-9,trans-11} \) CLA and \( \text{trans-10,cis-12} \) CLA are shown in Table 2. No significant effect of either isomer of CLA was seen on any of those markers, although there was a significant effect of dose for both fat mass and fat-free mass.

Comparison of the marginal means for fat mass suggested that mean fat mass was higher during supplementation with the lowest dose of CLA than at baseline and with the 2 higher doses of CLA (one-factor ANOVA repeated measures; \( P < 0.001 \)). Similarly, comparison of the marginal means for fat-free mass suggested that mean fat-free mass (expressed in kg only) was lower

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
</table>
| Effects of \( \text{cis-9,trans-11} \) conjugated linoleic acid (CLA) and \( \text{trans-10,cis-12} \) CLA on body weight and composition$^1$

|   | \( \text{cis-9,trans-11} \) CLA dose | \( \text{trans-10,cis-12} \) CLA dose | \( P \) for dose effect$^2$
| --- | --- | --- | ---
|   | 0 g/d | 0.59 g/d | 1.19 g/d | 2.38 g/d | 0 g/d | 0.63 g/d | 1.26 g/d | 2.52 g/d | 0 g/d | 0.63 g/d | 1.26 g/d | 2.52 g/d |
| Body mass (kg) | 77.20 ± 1.56 | 76.92 ± 1.44 | 77.94 ± 1.59 | 77.53 ± 1.66 | 77.75 ± 1.51 | 77.56 ± 1.50 | 78.33 ± 1.61 | 78.08 ± 1.52 | 77.75 ± 1.51 | 77.56 ± 1.50 | 78.33 ± 1.61 | 78.08 ± 1.52 |
| BMI (kg/m$^2$) | 24.19 ± 0.39 | 24.42 ± 0.37 | 24.54 ± 0.39 | 24.50 ± 0.43 | 24.46 ± 0.40 | 24.35 ± 0.41 | 24.67 ± 0.43 | 24.60 ± 0.40 | 24.46 ± 0.40 | 24.35 ± 0.41 | 24.67 ± 0.43 | 24.60 ± 0.40 |
| Body fat (%) | 17.55 ± 0.72 | 19.08 ± 0.78 | 18.47 ± 0.72 | 17.80 ± 0.74 | 17.97 ± 0.70 | 18.86 ± 0.73 | 18.43 ± 0.69 | 18.62 ± 0.72 | <0.01 | 17.97 ± 0.70 | 18.86 ± 0.73 | 18.43 ± 0.69 | 18.62 ± 0.72 | <0.01 |
| Fat mass (kg) | 15.54 ± 0.66 | 16.44 ± 0.70 | 15.35 ± 0.73 | 14.87 ± 0.75 | 15.69 ± 0.64 | 16.53 ± 0.67 | 15.98 ± 0.66 | 15.75 ± 0.68 | <0.01 | 15.69 ± 0.64 | 16.53 ± 0.67 | 15.98 ± 0.66 | 15.75 ± 0.68 | <0.01 |
| Fat-free mass (%) | 81.36 ± 0.59 | 80.04 ± 0.67 | 81.13 ± 0.65 | 81.75 ± 0.66 | 81.19 ± 0.58 | 80.18 ± 0.59 | 80.75 ± 0.58 | 80.81 ± 0.63 | <0.01 | 81.19 ± 0.58 | 80.18 ± 0.59 | 80.75 ± 0.58 | 80.81 ± 0.63 | <0.01 |
| Fat-free mass (kg) | 63.15 ± 1.03 | 61.7 ± 0.97 | 63.05 ± 1.08 | 63.14 ± 1.11 | 62.73 ± 1.01 | 61.84 ± 0.96 | 62.93 ± 1.05 | 62.76 ± 1.00 | <0.001 | 62.73 ± 1.01 | 61.84 ± 0.96 | 62.93 ± 1.05 | 62.76 ± 1.00 | <0.001 |

$^1$ All values are \( \bar{x} \) ± SEM, \( n = 39–49 \) subjects. There was no effect of isomer and no isomer × dose interaction on body fat, fat mass, or fat-free mass (by % or kg). The marginal means for body fat, fat mass, and fat-free mass (kg) for the two isomers combined across doses were significantly different, \( P < 0.001 \) (one-factor repeated-measures ANOVA). For all 3 variables, the effect of the lowest CLA dose was significantly different from the effect at baseline and the higher doses, \( P < 0.01 \) (Tukey tests).

$^2$ Two-factor repeated-measures ANOVA.
Effects of cis-9,trans-11 conjugated linoleic acid and trans-10,cis-12 conjugated linoleic acid on plasma lipids

No differences were seen in plasma triacylglycerol, NEFA, cholesterol, HDL-cholesterol, or LDL-cholesterol concentrations between isomer groups at baseline (Table 3). A significant effect of isomer on plasma triacylglycerol concentration (P < 0.05) was seen, but no significant effect was seen of dose and no isomer × dose interaction. Comparison of the marginal means for plasma triacylglycerol concentration indicated a significant difference between the effects of the 2 isomers (paired t test; P < 0.05), whereby the mean plasma triacylglycerol concentration was higher during supplementation with trans-10,cis-12 CLA than with cis-9,trans-11 CLA.

Significant effects were seen of both isomer and dose of CLA on plasma total cholesterol concentration (P < 0.05 and P < 0.02, respectively), but no isomer × dose interaction was seen (Table 3). Similarly, a significant effect was seen of both isomer and dose of CLA on plasma LDL-cholesterol concentration (P < 0.01 and P < 0.05, respectively) but no isomer × dose interaction (Table 3). No effect was seen of CLA on plasma HDL-cholesterol concentration (Table 3). No significant effects were seen of isomer or dose on plasma NEFA concentration, although there was a significant isomer × dose interaction (P < 0.05; Table 3).

A significant effect was seen of isomer on the LDL:HDL cholesterol (P < 0.01), but no effect of dose and no isomer × dose interaction (Table 3). The marginal means for LDL:HDL and total:HDL cholesterol differed significantly between isomers (P < 0.01, paired t test) and for each isomer compared with baseline (P < 0.02, paired t test). The opposite effects of the 2 CLA isomers on this outcome is clearly shown in Figure 2; the cis-9,trans-11 CLA isomer decreased the ratio relative to baseline, whereas the trans-10,cis-12 CLA isomer increased it.

Plasma glucose and insulin concentrations and assessment of insulin resistance

No effect was seen of isomer or dose of CLA on plasma insulin concentration or on indexes of insulin resistance and insulin sensitivity, HOMA-IR and revised QUICKI (Table 4). A significant effect was seen of isomer on plasma glucose concentration (P < 0.05), although there was no effect of dose and no
isomer X dose interaction. Comparison of the marginal means for plasma glucose indicated a significant difference between the effects of the 2 isomers (paired t test; \( P < 0.02 \)), whereby the mean plasma glucose was higher during supplementation with trans-10,cis-12 CLA than with cis-9,trans-11 CLA.

**DISCUSSION**

Supplementation with highly enriched cis-9,trans-11 CLA or trans-10,cis-12 CLA had no effect on body weight or BMI in healthy male volunteers in the present study. Although there appeared to be a slight increase in fat mass at the lowest dose of CLA compared with baseline, this marker returned to baseline values at the 2 higher doses of CLA. No clear explanation is available for the increase in fat mass at the lowest dose, but the return to baseline values is suggestive of an adaptational effect. It was considered unlikely to be a result of a lack of compliance toward the latter part of the intervention period because the fatty acid composition of plasma phospholipids showed a strong dose dependence (24). We, therefore, conclude that CLA supplementation had no consistent dose-dependent effect on body fat or fat-free mass.

The results are in general agreement with 4 other studies conducted in healthy adults, in which a mixture of CLA isomers had no effect on body weight or composition (12–15). One study reported a modest reduction in fat mass during CLA supplementation; subjects receiving 1.4 g/d CLA for 4 wk showed a significant reduction in fat mass compared with a lower intake of CLA (0.7 g/d), but it was not significantly different from the placebo group or from baseline (17). Nevertheless, the researchers concluded that there was a fat-lowering effect of CLA in healthy humans (17). Another study reported that 4.2 g/d CLA for 12 wk promoted a significant reduction in fat mass (−3.8%) compared with the control group (olive oil), with no change in body weight or BMI in healthy subjects (18). A reduction in fat mass was also reported in CLA-treated obese subjects (supplemented with 1.7, 3.4, 5.1, or 6.8 g/d CLA) compared with a placebo treatment, although it is important to note that the effect was statistically significant only at doses of 3.4 and 6.8 g/d (16). Thus, although this study used higher doses of CLA than the current study, it also failed to demonstrate dose-dependent effects of CLA on body composition. Furthermore, the CLA preparation contained a mixture of isomers. Animal studies suggested that the trans-10,cis-12 isomer has the most potent body fat-reducing properties (29, 30). Consequently, the lack of effect of CLA supplementation on body composition in human trials was sometimes explained as follows: the CLA supplements used in most trials contained a large mixture of isomers, and the trans-10,cis-12 isomer could be present at a concentration below the threshold necessary to elicit body composition changes. However, the present study demonstrates that even a fairly high dose of trans-10,cis-12 CLA does not affect body composition. One other study examined the effect of the trans-10,cis-12 isomer against a CLA mixture (50:50 mixture of trans-10,cis-12 and cis-9,trans-11 CLA isomers) on body composition in obese men with signs of metabolic syndrome (23). They reported a nonsignificant trend toward a decrease in body fat after supplementation with the trans-10,cis-12 isomer, whereas the CLA mixture had no effect (23). Overall, therefore, there is no conclusive evidence to suggest that consumption of either mixtures of CLA isomers or of highly enriched preparations of single CLA isomers results in a significant reduction in body composition.

Antiatherogenic properties were attributed to CLA (6, 7) and are believed to be a result, at least in part, of changes in lipoprotein metabolism (6). However, the reported effects of CLA on blood lipids are equivocal; this may once again be because previous studies used CLA preparations containing a mixture of isomers. One of the aims of the present study was to examine the effects of highly enriched preparations of cis-9,trans-11 CLA and trans-10,cis-12 CLA on blood lipids. There were significant effects of isomer on plasma triacylglycerol, cholesterol, and LDL-cholesterol concentrations; on the LDL cholesterol:HDL cholesterol, and on the percentage of change from baseline for the total cholesterol:HDL cholesterol. For plasma cholesterol and LDL-cholesterol concentrations, there was also a significant effect of dose, but there was no isomer X dose interaction. These data suggest a trend toward a decrease in plasma cholesterol and LDL cholesterol after supplementation with cis-9,trans-11 CLA but not trans-10,cis-12 CLA. For plasma triacylglycerol concentration, LDL cholesterol:HDL cholesterol, and percent of change from baseline for the total: HDL cholesterol, there was no significant effect of dose, so the marginal means for each isomer were compared. The marginal means for all 3 markers were significantly higher during supplementation with trans-10,cis-12 CLA. This finding highlighted a striking and consistent pattern. The pattern suggests opposing effects of the 2 isomers, with a detrimental effect of trans-10,cis-12 CLA relative to cis-9,trans-11 CLA, on the blood lipid profile, because LDL:HDL cholesterol and total:HDL cholesterol in particular are used as estimates of coronary risk (31, 32). To our knowledge, this is the first evidence that shows relative hyperlipidemic properties of trans-10,cis-12 CLA and hypolipidemic properties of cis-9,trans-11 CLA in humans. The lack of effect of dose suggests that this

### Table 4

Effects of cis-9,trans-11 conjugated linoleic acid (CLA) and trans-10,cis-12 CLA on plasma glucose, insulin, HOMA-IR, and revised QUICKI

<table>
<thead>
<tr>
<th>cis-9,trans-11 CLA dose</th>
<th>0 g/d</th>
<th>0.59 g/d</th>
<th>1.19 g/d</th>
<th>2.38 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.86 ± 0.09</td>
<td>4.89 ± 0.09</td>
<td>4.90 ± 0.12</td>
<td>4.75 ± 0.08</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>42.15 ± 4.03</td>
<td>38.59 ± 3.81</td>
<td>38.35 ± 3.73</td>
<td>39.32 ± 4.54</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.54 ± 0.01</td>
<td>1.41 ± 0.15</td>
<td>1.39 ± 0.13</td>
<td>1.43 ± 0.16</td>
</tr>
<tr>
<td>Revised QUICKI</td>
<td>0.47 ± 0.01</td>
<td>0.50 ± 0.01</td>
<td>0.48 ± 0.15</td>
<td>0.47 ± 0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>trans-10,cis-12 CLA dose</th>
<th>0 g/d</th>
<th>0.63 g/d</th>
<th>1.26 g/d</th>
<th>2.52 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.93 ± 0.08</td>
<td>4.99 ± 0.08</td>
<td>5.23 ± 0.10</td>
<td>4.89 ± 0.08</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>44.23 ± 3.79</td>
<td>43.02 ± 4.52</td>
<td>43.49 ± 4.28</td>
<td>45.14 ± 4.31</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.60 ± 0.14</td>
<td>1.64 ± 0.17</td>
<td>1.65 ± 0.16</td>
<td>1.61 ± 0.14</td>
</tr>
<tr>
<td>Revised QUICKI</td>
<td>0.47 ± 0.01</td>
<td>0.46 ± 0.01</td>
<td>0.47 ± 0.01</td>
<td>0.46 ± 0.01</td>
</tr>
</tbody>
</table>

* All values are \( \bar{x} \) ± SEM, \( n = 39–49 \) subjects. HOMA-IR, homeostasis model for insulin resistance; QUICKI, quantitative insulin sensitivity check index.

* Significance determined by two-way repeated-measures ANOVA.
influence could be exerted even at the lowest dose used in the study.

Although the current study did not include a placebo treatment, it is important to note that the statistics confirmed no period or treatment effects and no treatment-period interactions. Essentially, this finding demonstrates that regardless of the order of treatment, the effects of the treatments were the same in both arms of the study. Because each treatment lasted for 6 mo and the entire study’s duration was 13 mo, the effects of CLA on blood lipids are unlikely to represent a transient effect of time. Furthermore, there was no carry-over effect of either treatment. Also, although compliance was reported in the form of capsule counts in this study, we demonstrated an excellent dose-response relation for the incorporation of both isomers of CLA into plasma phospholipids and cholesteryl esters (24).

Reductions in total cholesterol and LDL-cholesterol concentrations in overweight subjects were observed after 12-wk supplementation with a CLA preparation (1.7–6.8 g/d) (16), and 4 wk of 0.7 g/d CLA resulted in tendency toward a decrease in triacylglycerol and total cholesterol concentrations in healthy subjects (17). However, neither of those studies used highly enriched preparations of cis-9,trans-11 CLA and trans-10,cis-12 CLA, making it impossible to elucidate which isomer was responsible for those effects. One study reported a 20% reduction in plasma triacylglycerol concentrations after an 8-wk supplementation with 3 g/d 50:50 mixture of cis-9,trans-11 CLA and trans-10,cis-12 CLA, whereas an 80:20 mixture did not have any effect (13), suggesting, indirectly, that the trans-10,cis-12 CLA is responsible for the triacylglycerol-lowering effect. That finding is in contrast with our findings, although the current study represents a more direct comparison of the effects of the 2 isomers.

The absence of a significant effect of CLA supplementation on the HDL-cholesterol concentration in the present study is in agreement with other relevant studies performed in normal-weight subjects (18, 20). However, it contrasts with the study of Riseras et al (23), which demonstrated that pure trans-10,cis-12 CLA decreased HDL-cholesterol concentration in obese subjects (23). Nevertheless, both the present study and that of Riseras et al (23) suggest that the trans-10,cis-12 isomer could have some adverse effects on cardiovascular risk factors.

The current study did not identify any effect of CLA on plasma insulin concentration, insulin resistance (HOMA-IR), or insulin sensitivity (revised QUICKI). For plasma glucose concentrations, marginal means were significantly higher during supplementation with the trans-10,cis-12 CLA than with the cis-9,trans-11 CLA, suggesting that the trans-10,cis-12 CLA increased blood glucose relative to the cis-9,trans-11 CLA. However, this effect was insufficient to modify the degree of insulin resistance or insulin sensitivity, which suggests that the increase in plasma glucose concentration might not be clinically relevant. Riseras et al (23) observed detrimental effects of 3.4 g/d trans-10,cis-12 CLA on insulin sensitivity in obese subjects with metabolic syndrome. However, this finding was not verified in healthy subjects in the current study or in previous human studies that used CLA mixtures (13, 18, 33).

In conclusion, neither of the CLA isomers affected body weight, body fat, or fat-free mass in healthy normal-weight subjects. There were divergent effects of cis-9,trans-11 CLA and trans-10,cis-12 CLA on some aspects of the blood lipid profile, which suggested a relative beneficial influence of the cis-9,trans-11 isomer and a detrimental influence of the trans-10,cis-12 isomer. However, no evidence was found that either of the CLA isomers adversely influenced insulin resistance. Use of CLA supplements containing high proportions of trans-10,cis-12 CLA should be considered with caution. Further studies to explore the mechanisms underlying the differential actions of the 2 isomers of CLA on blood lipids are warranted.

PCC, PY, CMW, and RFG designed the study and supervised the experimental work. ST, GCB, SK, TB, JIR, and ELJ screened, recruited, and sampled the volunteers and conducted the experimental work. ST, GCB, and PY analyzed the data. ST and PY wrote the manuscript, with input from all authors. None of the authors had any financial or personal interest in any company or organization sponsoring the research, including advisory board affiliations.

REFERENCES