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Postgraduate Symposium

The effects of conjugated linoleic acid on human health-related outcomes

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Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. CLA has received considerable attention as a result of animal experiments that report anti-carcinogenic, anti-atherogenic and anti-diabetic properties, and modulation of body composition and immune function. Several studies of CLA supplementation in human subjects have now been published, but in contrast to animal studies there has been marked variation between reports on the health-related outcomes.

The consensus from seventeen published studies in human subjects is that CLA does not affect body weight or body composition. Some detrimental effects of the trans-10,cis-12 CLA isomer have also been reported in terms of altered blood lipid composition and impaired insulin sensitivity. Finally, CLA has only limited effects on immune functions in man. However, there have been reports of some interesting isomer-specific effects of CLA on the blood lipid profile, but not on immune function. These isomer-specific effects need further investigation. Until more is known, CLA supplementation in man should be considered with caution.


Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometric isomers of linoleic acid (18:2) in which the two double bonds are conjugated, i.e. contiguous, unlike the double bonds in linoleic acid that are separated by a methylene group. CLA isomers are mainly present in ruminant animal fat, dairy products and partly-hydrogenated vegetable oils. Cis-9,trans-11 CLA is the main isomer in the human diet, accounting for >90% of the total CLA intake (Lawson et al. 2001), and is formed in the rumen as an intermediate in the microbial biohydrogenation of linoleic acid to stearic acid and endogenously in mammary tissue from trans-vaccenic acid, a precursor of rumen origin (Griinari & Bauman, 1999). Although diet is the major source of CLA in man, there is no systematic database for the CLA content of foods, and, because of the difficulties in the chromatographic separation of the individual isomers, limited data are available on the isomeric distribution of CLA isomers in food (Sebedio et al. 1999). Total CLA intake has been estimated to be between 52 and 137 mg/d for men and women in the USA, and to average 430 and 350 mg/d for German men and women respectively (McGuire et al. 1999). Over the last 10 years there has been increasing interest in CLA, as feeding a mixture of CLA isomers to laboratory animals has been reported to alter tumour growth induced by chemicals (Ip et al. 1991, 1994, 1995, 1999; Chew et al. 1997; Thompson et al. 1997; Belury, 2002), atherogenesis (Lee et al. 1994; Nicolosi et al. 1997), diabetes (Houseknecht et al. 1998), body composition (Park et al. 1997, 1999; West et al. 1998; de Deckere et al. 1999; DeLany et al. 1999; Azain et al. 2000; DeLany & West, 2000; Gavino et al. 2000; Stangl, 2000; Tsuboyama-Kasaoka

Abbreviations: CLA, conjugated linoleic acid; CRP, C-reactive protein; PBMC, peripheral blood mononuclear cells.

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et al. (2000) and immune cell functions (Cook et al. 1993; Miller et al. 1994; Turek et al. 1997, 1998; Sugano et al. 1998; Hayek et al. 1999; Bassaganya-Riera et al. 2001, 2002; Kelley et al. 2002; Yamasaki et al. 2003). Most of the published studies have used a mixture of CLA isomers containing the two major forms, cis-9,trans-11 CLA and trans-10,cis-12 CLA, in approximately equal amounts and a number of minor isomers at considerably lower levels. However, emerging evidence indicates that the numerous biological effects are a result of the separate actions of the cis-9,trans-11 and trans-10,cis-12 isomers (Pariza et al. 2000). The present review will focus on human studies and will attempt to define the extent to which the health effects of CLA relating to body composition, blood lipids, insulin sensitivity, immune function and inflammation reported in animal studies also occur in human subjects.

Conjugated linoleic acid and body composition

The body-fat-lowering effect of CLA observed in experimental animals has led to the possibility that CLA could be used as a tool in body-weight management in human subjects. Seventeen studies in human subjects have been published so far, but the results appear to be less promising than was expected (Calder, 2002; Kelley & Erickson, 2001; Kreider et al. 2002; Hayek et al. 2004; Tricon et al. 2003). Eight of these studies were conducted in subjects with normal body weight, whereas the other nine were conducted in overweight or obese subjects (Table 1). The majority of these studies have reported the effects of CLA on fairly healthy subjects. However, three of the studies investigated a population of men with signs of the metabolic syndrome (Riserus et al. 2001, 2002b, 2004a), while another used patients with type 2 diabetes (Belury et al. 2003). Most of the studies were done in free-living subjects and were not strictly controlled for nutrient and energy intake. Only in the study by Zambell et al. (2000) were the subjects confined to a metabolic unit (for 94 d) and matched to controls for food intake. The amount of CLA consumed in the various studies ranged from 0.7 g/d to 6.8 g/d. The CLA consumed was normally a 1:1 (w/w) mixture of the cis-9,trans-11 and trans-10,cis-12 CLA isomers (Blankson et al. 2000; Mougios et al. 2001; Riserus et al. 2001; Smedman & Vessby, 2001; Noone et al. 2002; Belury et al. 2003; Petridou et al. 2003; Gaullier et al. 2004), except for four studies that used a CLA preparation containing almost exclusively the trans-10,cis-12 isomer or the cis-9,trans-11 isomer (Riserus et al. 2002b, 2004b; Malpuech-Brugere et al. 2004; Tricon et al. 2004a). However, the earliest studies employed Tonalin (Natural ASA, Hovedbygda, Norway) capsules, a mixture of small amounts of several different CLA isomers in which trans-10,cis-12 and cis-9,trans-11 CLA isomers each represent about 200 mg/g (Berven et al. 2000; Thom et al. 2001; Zambell et al. 2001; Kreider et al. 2002). CLA was administered in the form of capsules, except in the study of Malpuech-Brugere et al. (2004) in which a dairy-based drink with added synthetic CLA was used.

CLA has not been demonstrated to have a marked effect on body weight or BMI (Table 1), although in one study reductions in body weight were observed in patients with type 2 diabetes mellitus receiving a supplement containing 6 g cis-9,trans-11 and trans-10,cis-12 CLA isomers (50:50, w/w)/d for 8 weeks (Belury et al. 2003). In addition, an inverse correlation was observed between body-weight change and plasma concentration of trans-10,cis-12 CLA (r = -0.4309; P < 0.05; Belury et al. 2003; Table 1). No correlation was found between body-weight change and plasma concentration of the cis-9,trans-11 isomer (Belury et al. 2003).

Conjugated linoleic acid and body composition in normal-weight subjects

Four human studies conducted in healthy normal-weight subjects have not demonstrated an effect of CLA on body fat mass (Zambell et al. 2001; Kreider et al. 2002; Petridou et al. 2003; Tricon et al. 2004b), while three studies have reported a modest reduction in fat mass after CLA supplementation (Mougios et al. 2001; Smedman & Vessby, 2001; Thom et al. 2001). However, although Mougios et al. (2001) have reported that healthy volunteers receiving 1.4 g CLA/d for 4 weeks have a marked decrease in fat mass and percentage body fat compared with volunteers receiving a lower intake of CLA (0.7 g/d), fat mass and percentage body fat values were not found to be significantly different from those for the placebo group or from baseline values (Table 1). The overall conclusion from this study must be that CLA, at the doses used, has little effect on body fatness (Mougios et al. 2001). Thus, only two studies report a reduction in body fat in healthy subjects (Smedman & Vessby, 2001; Thom et al. 2001).

It is of particular importance to address the effects of specific highly-purified isomers of CLA, rather than mixtures of CLA isomers, as it is possible that different isomers have different biological effects. Tricon et al. (2004b) have examined the effects of highly-enriched cis-9,trans-11 and trans-10,cis-12 preparations, each at three doses, on body composition in healthy males. Subjects (n 49) consumed one, two or four capsules (80–85 % pure CLA in a triacylglycerol form) daily, providing 0.59, 1.19 or 2.38 g cis-9,trans-11 CLA/d and 0.63, 1.26 or 2.52 g trans-10,cis-12 CLA/d respectively, for three consecutive 8-week periods (Fig. 1). The objective of the study was to identify for the first time the dose–response relationship between the two main CLA isomers and indices of body composition measured by bioelectrical impedance analysis. No significant effect on body weight, BMI, fat mass and fat-free mass was found for either isomer of CLA at any dose (Tricon et al. 2004b). The results are in general agreement with other studies conducted in healthy adults, in which a mixture of CLA isomers was found to have no effect on body weight or composition (Zambell et al. 2000; Kreider et al. 2002; Noone et al. 2002; Petridou et al. 2003). Animal studies have suggested that the trans-10,cis-12 isomer has the most potent body-fat-reducing properties (Park et al. 1999; Givino et al. 2000). Consequently, the lack of effect of CLA supplementation on body composition in human trials has sometimes been explained by the fact that the CLA supplements used in most trials have contained a mixture of isomers and the trans-10,cis-12 isomer may have been
present at a level below the threshold necessary to elicit body composition changes. However, the study by Tricon et al. (2004b) demonstrates that even a fairly high dose of trans-10,cis-12 CLA does not affect body composition in healthy subjects. Overall, therefore, there is no conclusive evidence to suggest that consumption of either a mixture of CLA isomers or of highly-enriched preparations of single CLA isomers results in a marked alteration in body composition in normal-weight subjects.

**Conjugated linoleic acid and body composition in overweight and obese subjects**

A summary of studies of the effects of CLA on body composition in overweight and obese subjects is given in Table 1. Three of seven studies have demonstrated that CLA supplementation has no effect on body composition in such subjects (Berven et al. 2000; Malpuech-Brugere et al. 2004; Riserus et al. 2004b). There are no effects of pure cis-9,trans-11 CLA or trans-10,cis-12 CLA (given in a food matrix) on body fat mass in overweight subjects after 18 weeks of supplementation (Malpuech-Brugere et al. 2004). In the study by Berven et al. (2000), 3.4 g CLA/d was reported to decrease mean body weight by 1.1 kg and mean BMI by 0.4 kg/m² after 12 weeks of supplementation in overweight and obese subjects. However, the overall treatment effect of CLA was not found to be significant. Finally, 3 months of supplementation with 3 g pure cis-9,trans-11 CLA was not found to affect body composition in twenty-five abdominally-obese men (Riserus et al. 2004b), suggesting that cis-9,trans-11 CLA has no anti-obesity effects. This result is in accord with evidence in mice (Park et al. 1999), which suggests that trans-10,cis-12 CLA isomer is the anti-adipogenic isomer (Pariza et al. 2001).

On the other hand, Blankson et al. (2000) have reported that overweight or moderately-obese, but otherwise healthy, subjects supplementing their diet with 3-4 or 6-8 g of a CLA preparation (Tonalin) daily for 12 weeks experience greater losses in fat mass (determined by dual-energy X-ray absorptiometry; –1.7 and –1.3 kg respectively) when compared with the placebo (+1.8 kg). However, no effects were observed when subjects were administered 1.7 or 5.1 g CLA/d, suggesting no clear dose–response effect. The authors have claimed that a CLA intake of 3-4 g/d reduces body fat. However, this conclusion is substantially weakened because the decrease in body fat was not found to be significant in the group administered 5-1 g CLA/d. Furthermore, lean body mass was reported to increase markedly only in the group administered 5-8 g CLA/d, which was the group that reported the maximum increase in the period of time (h) spent undertaking intensive exercise (Blankson et al. 2000). In a second study of a group of abdominally-obese men supplemented with 4-2 g CLA/d for 4 weeks a significant mean decrease in sagittal abdominal diameter of 6 mm was demonstrated in the CLA group when compared with the control group (Riserus et al. 2001). Decreases in the waist: hip ratio and waist circumference were also observed within the CLA group, but these changes were not significantly different from those of the control group. Furthermore, in the studies by Riserus et al. (2001) and Blankson et al. (2000), the changes in body fat mass are within the prediction errors for the methods used (Kelley & Erickson, 2003). In a third study Gaullier et al. (2004) have examined, for the first time, the long-term effect of 3 g CLA (in NEFA or triacylglycerol forms)/d in overweight subjects (Table 1). They have reported that 1 year of supplementation with CLA (in either form) markedly lowers body fat mass, and that CLA (in the NEFA form) increases lean body mass compared with a placebo (Gaullier et al. 2004). However, the authors changed their techniques for measuring body composition halfway through the study and have reported a reduction in energy intake in all groups, particularly in the two CLA-supplemented groups. The authors have also reported that the best responders to CLA (≥4.5% body fat mass reduction) are women and subjects with a higher BMI at baseline (Gaullier et al. 2004). This finding is quite interesting, as none of the studies looking at the effects of CLA on body composition have stratified for gender or BMI.

Recently, Riserus et al. (2002b) have reported that the trans-10,cis-12 CLA isomer (approximately 2 g/d) for 12 weeks is responsible for a marked trend towards a decrease in body fat, sagittal abdominal diameter, waist girth, BMI and weight in sixty obese men with signs of the metabolic syndrome, whereas only sagittal abdominal diameter and body fat decrease after a CLA mixture (trans-10,cis-12 and cis-9,trans-11 CLA isomers; 50:50, w/w). These data would suggest that the trans-10,cis-12 CLA is the active isomer in terms of weight-loss (Riserus et al. 2002b; Belury et al. 2003), although this finding was not confirmed in the two studies that investigated the effects of the pure CLA isomers (Malpuech-Brugere et al. 2004; Tricon et al. 2004b). Furthermore, no significant differences were observed between groups (control v. CLA mixture v. trans-10,cis-12 CLA) for any of the body composition variables measured after 12 weeks (Riserus et al. 2002b).

Collectively, results from human studies relating to the effects of CLA on body composition in overweight and obese subjects are inconsistent. Four of the seven studies indicate a possible reduction in body fat, whereas the others report no change. However, it is likely that the changes reported in at least some studies were the result of confounding variables such as food intake, exercise and the prediction errors for the methods used. Furthermore, the effects are much smaller than those observed in animals and the weight of evidence does not support a role for CLA in decreasing body fat in man.

**Conjugated linoleic acid and body-weight regain**

Most of the animal experiments in which CLA has been found to decrease fat deposition have been conducted with growing animals. It is important to emphasize that in many animal models dietary CLA induces a decrease in body fat without decreasing body weight (Park et al. 1997). Thus, in most animal models the decrease in body fat appears to be related mostly to a reduction in body fat accretion (West et al. 1998; Pariza, 2004). However, the published human trials described earlier were all designed to test the
<table>
<thead>
<tr>
<th>Amount of CLA (g/d)</th>
<th>Form of CLA</th>
<th>Placebo</th>
<th>Duration</th>
<th>Subjects</th>
<th>Effect of CLA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>Tonalin*</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Sixty overweight or obese males and females Age &gt;18 years</td>
<td>No effect on body weight or BMI</td>
<td>Berven et al. (2000)</td>
</tr>
<tr>
<td>1.7, 3.4, 5.1 and 6.8</td>
<td>Cis-9:\ trans-11\ and trans-10, cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Sixty overweight or obese but healthy males and females</td>
<td>Decrease in body fat mass in 3.4 and 6.8 g/d groups</td>
<td>Blankson et al. (2000)</td>
</tr>
<tr>
<td>3.9</td>
<td>Tonalin*</td>
<td>Sunflower oil</td>
<td>9 weeks in a metabolic suite</td>
<td>Seventeen healthy females Age 20–41 years</td>
<td>Decrease in body fat mass in the 3.4 g/d group</td>
<td>Zambell et al. (2000)</td>
</tr>
<tr>
<td>0.7 then 1.4</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w)</td>
<td>Soyabean oil</td>
<td>4 weeks then 4 weeks</td>
<td>Forty-four healthy males and ten healthy females Age 19–24 years</td>
<td>Decrease in body fat mass during the high CLA intake compared with the low CLA intake</td>
<td>Mougios et al. (2001)</td>
</tr>
<tr>
<td>4.2</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>4 weeks</td>
<td>Twenty-four abdominally-obese males with signs of the metabolic syndrome Age 39–64 years</td>
<td>Decrease in SAD</td>
<td>Riserus et al. (2001)</td>
</tr>
<tr>
<td>4.2</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Twenty-two healthy males and twenty-six healthy females Age 23–63 years</td>
<td>Decrease in body fat</td>
<td>Smedman &amp; Vessby (2001)</td>
</tr>
<tr>
<td>1.8</td>
<td>Tonalin*</td>
<td>Hydrogel</td>
<td>12 weeks</td>
<td>Twenty healthy exercising males and females Age 18–30 years</td>
<td>No effect on BMI or body weight</td>
<td>Thom et al. (2001)</td>
</tr>
<tr>
<td>3.0</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w) v. cis-9, trans-11 and trans-10, cis-12 (80:20, w/w)</td>
<td>Linoleic acid</td>
<td>8 weeks</td>
<td>Eighteen healthy males and thirty-three healthy females Age 30–60 years</td>
<td>No effect on body weight or BMI</td>
<td>Noone et al. (2002)</td>
</tr>
<tr>
<td>3.4</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w) v. trans-10, cis-12</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Sixty abdominally-obese males with signs of the metabolic syndrome Age 35–65 years</td>
<td>No effect on body composition</td>
<td>Riserus et al. (2002b)</td>
</tr>
<tr>
<td>6.0</td>
<td>Tonalin*</td>
<td>Olive oil</td>
<td>28 d</td>
<td>Twenty-three experienced resistance-trained males Age 23 years (mean)</td>
<td>Decrease in weight, BMI, waist:hip ratio or SAD</td>
<td>Kreider et al. (2002)</td>
</tr>
<tr>
<td>2.1</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w)</td>
<td>Soyabean oil</td>
<td>45d</td>
<td>Sixteen healthy females Age 19–24 years</td>
<td>No effect on body composition</td>
<td>Petridou et al. (2003)</td>
</tr>
<tr>
<td>6.0</td>
<td>Cis-9, trans-11 and trans-10, cis-12 (50:50, w/w)</td>
<td>Safflower oil</td>
<td>8 weeks</td>
<td>Twenty-one subjects with type II diabetes</td>
<td>Levels of plasma trans-10, cis-12 CLA, but not cis-9, trans-11 CLA, were inversely associated with body weight</td>
<td>Belury et al. (2003)</td>
</tr>
</tbody>
</table>
hypothesis that CLA ingestion will lower the amount of accumulated body fat in adult subjects (Table 1). Only Kamphuis et al. (2003a,b) have approached the issue differently, examining the effects of two doses of CLA administered after weight loss on body-weight and body-fat regain (Table 1). Overweight subjects were first submitted to a 3-week very-low-energy weight-loss diet and then supplemented with 1.8 or 3.6 g CLA (as Tonalin/d or a placebo for a 13-week intervention period during which they ate ad libitum (Kamphuis et al. 2003a). Subjects taking CLA (at either dose) were found to exhibit greater regain of fat-free mass relative to control subjects, accompanied by an increase in RMR (Kamphuis et al. 2003a). However, CLA was not found to affect percentage body-weight regain (Kamphuis et al. 2003a).

Interestingly, measures of appetite (hunger, satiety and fullness) were also observed to be favourably and dose-dependently affected by CLA ingestion (Kamphuis et al. 2003b). This study is in accordance with animal studies suggesting that CLA might be most effective in controlling body fat accretion, rather than lowering the amount of accumulated body fat.

Conjugated linoleic acid and blood lipid concentrations

Several of the human studies described earlier have also reported the effects of CLA on plasma lipid concentrations. As for the reported body-fat-lowering effect of CLA in human subjects, the results appear to be inconsistent and less promising than expected (Table 2). The studies by Berven et al. (2000), Benito et al. (2001), Riserus et al. (2001), Smedman & Vessby (2001) and Petridou et al. (2003) do not show any marked effect of CLA (a mixture of the cis-9, trans-11 and trans-10,cis-12 isomers) on plasma total cholesterol, LDL- and HDL-cholesterol or triacylglycerol concentrations. Whilst these studies have shown that CLA may not affect lipoprotein metabolism in human subjects, other reports suggest a detrimental HDL-lowering effect of CLA mixtures (Blankson et al. 2000; Mougios et al. 2001; Riserus et al. 2002b; Gaullier et al. 2004). This lowering of HDL-cholesterol concentration by CLA appears to be more apparent in obese subjects (Blankson et al. 2000; Riserus et al. 2002b; Gaullier et al. 2004), raising some safety concerns about CLA supplementation. In the study by Gaullier et al. (2004), the decrease in HDL-cholesterol concentration was only observed when CLA was supplemented in the triacylglycerol form (Table 2) and was not considered by the authors to be of clinical importance. In the study by Riserus et al. (2002b), a randomized double-blind controlled trial in which abdominally-obese men were given daily 3.4 g CLA (isomer mixture) or purified trans-10, cis-12 CLA, or a placebo for 12 weeks, it was demonstrated that the trans-10,cis-12 isomer, but not the CLA mixture, is responsible for a significant decrease in HDL-cholesterol concentration (−4%; \( P < 0.01 \), unpaired \( t \) test) coupled with a non-significant tendency to increased VLDL-triacylglycerol concentrations. The findings of this last study seem to suggest that the trans-10,cis-12 CLA is the isomer responsible for impairment of the blood lipid
effects of CLA (Houseknecht et al. Studies in animal models have reported anti-diabetic findings it has been speculated that CLA could potentially be useful for the treatment and prevention of type 2 diabetes and metabolic syndrome. However, of the few studies published so far, none has reported any marked effect of CLA supplementation on fasting blood glucose or plasma insulin concentrations (Medina et al. 2000; Smedman & Vessby, 2001; Noone et al. 2002). Furthermore, some researchers have recently raised concerns about the potential safety of CLA for human subjects in terms of insulin resistance (Riserus et al. 2002b, 2004a; Larsen et al. 2003). Riserus et al. (2002b) have conducted the only study to date that has tested the effect of CLA using direct insulin-sensitivity measurements. A group of sixty abdominally-obese men with signs of the metabolic syndrome were randomly assigned to one of three supplements containing 3.4 g CLA isomer mixture or the purified trans-10,cis-12 isomer/d or a placebo for 12 weeks. The trans-10,cis-12 isomer was reported to markedly decrease insulin sensitivity (as determined by an intravenous glucose tolerance test) and increase fasting plasma glucose concentration compared with the placebo. More recently, this research group has also conducted a randomized double-blind placebo-controlled study in twenty-five abdominally-obese men who received 3 g pure cis-9,trans-11 CLA/d or a placebo (olive oil) for 3 months (Riserus et al. 2004b). They observed that cis-9,trans-11 CLA also decreases insulin sensitivity (by 15%; P < 0.05) compared with the placebo (Riserus et al. 2004b). This report is the first to suggest some detrimental effects of the cis-9,trans-11 CLA isomer on human health. As both CLA isomers appear to decrease insulin sensitivity it is, however, quite surprising that such an effect was not observed when a 50:50 (w/w) mixture of CLA isomers was given as a supplement (Riserus et al. 2002b). In another study (Fig. 1) Tricon et al. (2004b) have suggested that the trans-10,cis-12 CLA increases fasting blood glucose concentration relative to the cis-9,trans-11 CLA isomer in healthy males. However,

<table>
<thead>
<tr>
<th>Trans-10,cis-12 CLA isomer at three doses</th>
<th>Cis-9,trans-11 CLA isomer at three doses</th>
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<tbody>
<tr>
<td>0.63 g/d 1.26 g/d 2.52 g/d W/O 0.63 g/d 1.26 g/d 2.52 g/d</td>
<td>0.59 g/d 1.19 g/d 2.38 g/d W/O 0.59 g/d 1.19 g/d 2.38 g/d</td>
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</table>

**Fig. 1.** Design of the study conducted by Burdge et al. (2004) and Tricon et al. (2004a,b). The subjects provided a fasting blood sample at baseline and then every 8 weeks during conjugated linoleic acid (CLA) supplementation. On each arm of the study the subjects consumed 0.59, 1.19 and 2.38 g cis-9,trans-11 CLA/d or 0.63, 1.26 and 2.52 g trans-10,cis-12 CLA/d in consecutive 8-week periods. The two arms of the study were separated by a 6-week washout period (W/O). Body weight and composition were also determined alongside each 8-week visit for blood sampling. (From Tricon et al. 2004b; reproduced with permission from American Journal of Clinical Nutrition.)

**Conjugated linoleic acid and insulin sensitivity**

Studies in animal models have reported anti-diabetic effects of CLA (Houseknecht et al. 1998; Ryder et al. 2001; Evans et al. 2002). Thus, on the basis of these reported findings it has been speculated that CLA could potentially be useful for the treatment and prevention of type 2 diabetes and metabolic syndrome. However, of the few studies published so far, none has reported any marked effect of CLA supplementation on fasting blood glucose or plasma insulin concentrations (Medina et al. 2000; Smedman & Vessby, 2001; Noone et al. 2002). Furthermore, some researchers have recently raised concerns about the potential safety of CLA for human subjects in terms of insulin resistance (Riserus et al. 2002b, 2004a; Larsen et al. 2003). Riserus et al. (2002b) have conducted the only study to date that has tested the effect of CLA using direct insulin-sensitivity measurements. A group of sixty abdominally-obese men with signs of the metabolic syndrome were randomly assigned to one of three supplements containing 3.4 g CLA isomer mixture or the purified trans-10,cis-12 isomer/d or a placebo for 12 weeks. The trans-10,cis-12 isomer was reported to markedly decrease insulin sensitivity (as determined by an intravenous glucose tolerance test) and increase fasting plasma glucose concentration compared with the placebo. More recently, this research group has also conducted a randomized double-blind placebo-controlled study in twenty-five abdominally-obese men who received 3 g pure cis-9,trans-11 CLA/d or a placebo (olive oil) for 3 months (Riserus et al. 2004b). They observed that cis-9,trans-11 CLA also decreases insulin sensitivity (by 15%; P < 0.05) compared with the placebo (Riserus et al. 2004b). This report is the first to suggest some detrimental effects of the cis-9,trans-11 CLA isomer on human health. As both CLA isomers appear to decrease insulin sensitivity it is, however, quite surprising that such an effect was not observed when a 50:50 (w/w) mixture of CLA isomers was given as a supplement (Riserus et al. 2002b). In another study (Fig. 1) Tricon et al. (2004b) have suggested that the trans-10,cis-12 CLA increases fasting blood glucose concentration relative to the cis-9,trans-11 CLA isomer in healthy males. However,
Table 2. Published studies investigating the effect of conjugated linoleic acid (CLA) on plasma lipids in human subjects

<table>
<thead>
<tr>
<th>Amount of CLA (g/d)</th>
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<th>Placebo</th>
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<tr>
<td>1-7, 3-4, 5-1, and 6-8</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Sixty overweight or obese but healthy males and females</td>
<td>Decrease in total cholesterol, LDL- or HDL-cholesterol, or TAG</td>
<td>Blankson et al. (2000)</td>
</tr>
<tr>
<td>3-9</td>
<td>Tonalin*</td>
<td>Sunflower oil</td>
<td>9 weeks in a metabolic suite</td>
<td>Seventeen healthy females</td>
<td>No effect on total cholesterol, LDL- or HDL-cholesterol, or TAG</td>
<td>Benito et al. (2001)</td>
</tr>
<tr>
<td>0-7 then 1-4</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w)</td>
<td>Soyabean oil</td>
<td>4 weeks then 4 weeks</td>
<td>Fourteen healthy males and ten healthy females</td>
<td>Decrease in HDL-cholesterol during low dose</td>
<td>Mougios et al. (2001)</td>
</tr>
<tr>
<td>4-2</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>4 weeks</td>
<td>Twenty-four abdominally-obese males with signs of the metabolic syndrome</td>
<td>No effect on total cholesterol, LDL- or HDL-cholesterol, or TAG</td>
<td>Risenus et al. (2001)</td>
</tr>
<tr>
<td>4-2</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Twenty-seven healthy males and twenty-six healthy females</td>
<td>No effect on total cholesterol, LDL- or HDL-cholesterol, TAG or NEFA</td>
<td>Smedman &amp; Vessby (2001)</td>
</tr>
<tr>
<td>3-0</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w) v. cis-9,trans-11 and trans-10,cis-12 (80:20, w/w)</td>
<td>Linoleic acid-rich vegetable oil</td>
<td>8 weeks</td>
<td>Eighteen healthy males and thirty-three healthy females</td>
<td>Decrease in TAG with the 50:50 (w/w) isomer mix</td>
<td>Noone et al. (2002)</td>
</tr>
<tr>
<td>3-4</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w) CLA v. trans-10,cis-12 (50:50, w/w)</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Sixty abdominally-obese males with signs of the metabolic syndrome</td>
<td>Decrease in HDL-cholesterol with trans-10,cis-12 CLA</td>
<td>Risenus et al. (2002b)</td>
</tr>
<tr>
<td>2-1</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w)</td>
<td>Soyabean oil</td>
<td>45 d</td>
<td>Sixteen healthy females</td>
<td>Tendency to decreased HDL-cholesterol with the CLA mix</td>
<td>Petridou et al. (2003)</td>
</tr>
<tr>
<td>4-5</td>
<td>Cis-9,trans-11 and trans-10,cis-12 (50:50, w/w) as NEFA or TAG</td>
<td>Olive oil</td>
<td>1 year</td>
<td>Eighty overweight males and females</td>
<td>Decrease in HDL-cholesterol with TAG as TAG</td>
<td>Gauillier et al. (2004)</td>
</tr>
<tr>
<td>3-0</td>
<td>cis-9,trans-11</td>
<td>Olive oil</td>
<td>12 weeks</td>
<td>Twenty-five abdominally-obese males</td>
<td>No effect on total cholesterol, LDL-, HDL- or VLDL-cholesterol, or TAG</td>
<td>Risenus et al. (2004b)</td>
</tr>
<tr>
<td>0-59, 1-19, 2-38 (cis-9,trans-11: 0.63, 1-26, 2-52 (trans-10,cis-12)</td>
<td>Cis-9,trans-11 v. trans-10,cis-12</td>
<td>None (cross-over design)</td>
<td>8 weeks on each dose (doses increased sequentially; 6-week washout between isomers)</td>
<td>Forty-nine healthy males</td>
<td>Increased cholesterol:HDL-cholesterol, LDL-cholesterol:HDL-cholesterol and TAG with trans-10,cis-12 CLA relative to cis-9,trans-11 CLA</td>
<td>Tricon et al. (2004b)</td>
</tr>
</tbody>
</table>

TAG, triacylglycerol.

*Composition (mg/g): 226, trans-10,cis-12; 236, cis-11,trans-13, 17-6; cis-9,trans-11; 166, trans-8,cis-10; 77, trans-9,trans-11 and trans-10,trans-12 and 11-9 other CLA isomers.
Conjugated linoleic acid and immune function

There is very little information relating to the effects of CLA on immune and inflammatory outcomes in human subjects. A placebo-controlled metabolic unit study has been conducted in seventeen healthy women supplemented daily with 3·9 g CLA (Tonalin capsules consisting of minor isomers into PBMC, although the final concentration of each isomer was reported to be markedly lower than that in plasma phosphatidylcholine and cholesteryl ester fractions at each dose (Burdge et al. 2004). In terms of immune function, no effects were found of either isomer of CLA supplementation (Kelley et al. 2000). Fatty acid profiles of isolated peripheral blood mononuclear cells (PBMC) demonstrate an eightfold increase in CLA as a proportion of total fatty acids (from 0·12 mg total CLA/100 mg to 0·97 mg total CLA/100 mg); the largest increase is in the cis-11,trans-13 CLA isomer. CLA does not affect the production of eicosanoids (prostaglandin E₂ and leukotriene B₄) or cytokines (TNF-α, interferon-γ, IL-1 and -2) by PBMC after mitogen stimulation (Kelley et al. 2001). There are also no changes in markers of immunity (neutrophils:lymphocytes) in experienced resistance-trained males receiving 6 g CLA (as Tonalin capsules)/d for 28 d v. a placebo (olive oil; Kreider et al. 2002). In contrast, the recently-published results of a double-blinded intervention trial suggest that the individual isomers of CLA could have different effects on components of the immune system in human subjects (Albers et al. 2003). A mixture of cis-9,trans-11 and trans-10,cis-12 CLA (50:50, w/w), at a dose of 1·7 g CLA/d was found to result in a greater proportion of individuals producing a protective antibody titre (>10 IU/l) to hepatitis B vaccination, although the mean antibody titres do not differ between groups. This study was the first in which CLA has been shown to promote the humoral immune response in human subjects, as reflected by an increased seroprotection rate after vaccination. However, Kelley et al. (2000) and Kelley & Erickson (2003) question the interpretation that was based on the use of arbitrary thresholds for seroprotective titres. Furthermore, in the healthy subjects none of the other aspects of immune function measured (delayed hypersensitivity response, natural killer cell activity, lymphocyte proliferation and production of TNF-α, IL-1β, IL-6, IFN-γ, IL-2, IL-4 and prostaglandin E₂) were reported to be affected (Albers et al. 2003). In a separate study of the two isomeric mixtures of CLA (cis-9,trans-11 and trans-10, cis-12 CLA at 50:50 (w/w) and 80:20 (w/w), the 80:20 (w/w) mixture (3 g/d for 8 weeks) was found to markedly enhance peripheral blood lymphocyte proliferation in response to the T-cell mitogen phytohaemagglutinin, whereas treatment with the 50:50 (w/w) mixture markedly decreases concanavalin-induced proliferation (Roche et al. 2001). These findings would suggest that the supplement providing more of the cis-9,trans-11 CLA isomer promotes the cell-mediated immune response, whereas the supplement which has a greater amount of the trans-10,cis-12 CLA isomer attenuates the immune response (Roche et al. 2001). A recent study (Burgoe et al. 2004; Tricon et al. 2004a,b) has described for the first time the effect of consuming increasing amounts of highly-enriched preparations of cis-9,trans-11 and trans-10,cis-12 CLA on their incorporation into PBMC (Burgoe et al. 2004) and on immune function (Tricon et al. 2004a; Fig. 1). Both cis-9,trans-11 and trans-10,cis-12 CLA isomers were found to be incorporated in a dose-dependent manner into PBMC total lipids (r 0.285 and r 0.273 respectively; P < 0.0005) when consumed in the diet (Burgoe et al. 2004). No evidence was found for differential incorporation of these isomers into PBMC, although the final concentration of each isomer was reported to be markedly lower than that in plasma phosphatidylcholine and cholesteryl ester fractions at each dose (Burgoe et al. 2004). In terms of immune function, no effects were found of either isomer of CLA on PBMC subsets and on ex vivo cytokine production.

Fig. 2. Effects of cis-9,trans-11 conjugated linoleic acid (CLA): ■ and trans-10,cis-12 CLA (●●●) on the percentage change in total cholesterol:HDL-cholesterol (TC:HDL-C) from baseline. One, two or four capsules per d provided respectively 0·59, 1·19 or 2·38 g cis-9,trans-11 CLA/d or 0·63, 1·26 or 2·52 g trans-10,cis-12 CLA/d. Values are means with their standard errors represented by vertical bars for thirty-nine to forty-nine subjects. There was a significant effect of isomer (two-way ANOVA repeated measures, P < 0·01), but no significant effect of dose and no isomer × dose interaction. The marginal means for the percentage change in total cholesterol:HDL-cholesterol for the cis-9,trans-11 CLA treatments were significantly different from those for the trans-10,cis-12 CLA treatments (paired t test; P < 0·001). (From Tricon et al. 2004b; reproduced with permission from American Journal of Clinical Nutrition.)

This effect was found to be insufficient to modify the extent of insulin resistance or insulin sensitivity, calculated by the surrogate measures: the homeostasis model for insulin resistance (Matthews et al. 1985); the quantitative insulin-sensitivity check index (Katz et al. 2000). These findings would suggest that the detrimental effect of the trans-10, cis-12 CLA isomer on insulin sensitivity observed by Riserus et al. (2002b) may be of particular importance only in obese subjects with metabolic syndrome but may not be important in healthy subjects (Medina et al. 2000; Smedman & Vessby, 2001; Noone et al. 2002; Tricon et al. 2004b).
Conjugated linoleic acid and inflammation

C-reactive protein (CRP) is a marker of chronic subclinical inflammation, providing a sensitive indicator of underlying inflammation in the body, and levels are reported to be raised dramatically (≤100-fold) in infection and inflammation (Tracy, 1998). Several large epidemiological studies have reported that a high serum level of CRP is a strong independent predictor of future myocardial infarction and stroke in individuals without known CVD (Yudkin et al., 1999; Ridker, 2001). Concern about CLA-induced elevations in serum CRP levels has arisen from a study by Riserus et al. (2002a), who have investigated the effects of CLA in obese men with signs of the metabolic syndrome. They have reported that a supplement highly enriched in \( \text{trans}^{-10, \text{cis}^{-12}} \) CLA (3.4 g/d for 3 months) markedly increases CRP (+110%) compared with a placebo (olive oil).

CRP is an acute-phase protein, synthesized and released from the liver, under the influence of IL-6. Interestingly, the apparent \( \text{trans}^{-10, \text{cis}^{-12}} \)-CLA-induced serum CRP elevation is not accompanied by an increase in IL-6 levels (Riserus et al. 2002a). In the study by Tricon et al. (2004a; Fig. 1), no marked effects on serum CRP concentration were found for either CLA isomer, suggesting that CLA supplementation with doses of ≤25.2 g/d does not influence CRP in healthy subjects, but may enhance the already higher CRP levels in obese men.

Conclusions

Results from human studies relating to the effects of CLA on body composition, blood lipids, insulin resistance and immune function have been variable. The evidence from short-term studies in human subjects suggests that CLA supplementation does not decrease body weight and body fat. There is evidence that CLA isomers have marked biological effects, and there is accumulating evidence that the \( \text{trans}^{-10, \text{cis}^{-12}} \) CLA isomer may adversely influence human health, in particular concerning insulin sensitivity and blood lipids. More controlled studies in specific populations with purified isomers of CLA are needed and should be used to define the beneficial and detrimental effects of each individual CLA isomer. Until then CLA supplementation for human subjects should not be recommended.

Acknowledgements

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References


(Tricon et al. 2004a). However, this study has demonstrated for the first time a dose-dependent reduction in the activation of T lymphocytes (measured by cell surface expression of the early activation marker CD69) by both \( \text{cis}^{-9, \text{trans}^{-11}} \) and \( \text{trans}^{-10, \text{cis}^{-12}} \) CLA, which is inversely correlated with the proportions of both isomers in PBMC lipids (Tricon et al. 2004a). However, as the function of CD69 has not been fully characterized, the implications and relevance of the effects of CLA on lymphocyte activation are not clear. Interestingly, CLA does not exhibit isomer-specific effects in relation to lymphocyte activation. This finding is in contrast to the differential effects of the two CLA isomers on blood lipids reported for the same subjects (Tricon et al. 2004b).

It can be concluded from the few human studies published so far that CLA does not seem to be a strong modulator of immune function in human subjects. However, further studies are warranted to investigate a larger number of measurements of immune function involved in both the adaptive and innate responses. Furthermore, until definitive molecular evidence is available on the mechanism(s) of action of CLA, it will be difficult to use CLA in possible preventive and therapeutic applications.


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spleen lymphocytes is modulated in C57BL/6J mice by dietary cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid. Journal of Nutrition 133, 784–788.

