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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.

CLA: Body composition: Blood lipids: Insulin resistance: Immune function

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, 2003; Larsen *et al*. 2003; Terpstra, 2004). 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, 2002*b*, 2004*a*), 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*. 2002*b*, 2004*b*; Malpuech-Brugere *et al*. 2004; Tricon *et al*. 2004*a*). However, the earliest studies employed Tonalin (Natural ASA, Hovdebygda, 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*. 2004*b*), 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*. (2004*b*) 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*. 2004*b*). 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; Gavino *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

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present at a level below the threshold necessary to elicit body composition changes. However, the study by Tricon *et al*. (2004*b*) 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*. 2004*b*). 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 /m^2 after 12 weeks of supplementation in overweight and obese participants. However, the overall treatment effect of CLA was not found to be marked. Finally, 3 months of supplementation with 3 g pure *cis*-9,*trans*-11 CLA/d was not found to affect body composition in twenty-five abdominally-obese men (Riserus *et al*. 2004*b*), 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 6·8 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*. (2002*b*) 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*. 2002*b*; 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*. 2004*b*). 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*. 2002*b*).

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

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Table 1. Published studies investigating the effect of conjugated linoleic acid (CLA) on body composition in human subjects

Amount of CLA (g/d)	Form of CLA	Placebo	Duration	Subjects	Effect of CLA	Reference
3·4	Tonalin*	Olive oil	12 weeks	Sixty overweight or obese males and females Age >18 years	No effect on body weight or BMI	Berven *et al.* (2000)
1·7, 3·4, 5·1 and 6·8	*Cis*-9,*trans*-11–*trans*-10,*cis*-12 (50:50, w/w)	Olive oil	12 weeks	Sixty overweight or obese but healthy males and females	Decrease in body fat mass in 3·4 and 6·8 g/d groups No effect on body weight or BMI	Blankson *et al.* (2000)
3·9	Tonalin*	Sunflower oil	9 weeks in a metabolic suite	Seventeen healthy females Age 20–41 years	No effect on body weight, body fat mass or body lean mass	Zambell *et al.* (2000)
0·7 then 1·4	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Soyabean oil	4 weeks then 4 weeks	Fourteen healthy males and ten healthy females Age 19–24 years	Decrease in percentage body fat and fat mass during the high CLA intake compared with the low CLA intake	Mougios *et al.* (2001)
4·2	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Olive oil	4 weeks	Twenty-four abdominally-obese males with signs of the metabolic syndrome Age 39–64 years	Decrease in SAD	Riserus *et al.* (2001)
4·2	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Olive oil	12 weeks	Twenty-seven healthy males and twenty-six healthy females Age 23–63 years	Decrease in body fat No effect on body weight, BMI, waist:hip ratio or SAD	Smedman & Vessby (2001)
1·8	Tonalin*	Hydrogel	12 weeks	Twenty healthy exercising males and females Age 18–30 years	No effect on BMI or body weight Decrease in body fat	Thom *et al.* (2001)
3·0	*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)	Linoleic acid	8 weeks	Eighteen healthy males and thirty-three healthy females Age 31·6 years (mean)	No effect on body weight or BMI	Noone *et al.* (2002)
3·4	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w) *v.* *trans*-10,*cis*-12	Olive oil	12 weeks	Sixty abdominally-obese males with signs of the metabolic syndrome Age 35–65 years	No effect on body composition Decrease in weight, BMI, waist circumference, SAD and body fat in the *trans*-10,*cis*-12 isomer group Decrease in SAD and body fat in the CLA mixture group	Riserus *et al.* (2002*b*)
6·0	Tonalin*	Olive oil	28 d	Twenty-three experienced resistance-trained males Age 23 years (mean)	No effect on weight, body fat or lean mass	Kreider *et al.* (2002)
2·1	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Soyabean oil	45 d	Sixteen healthy females Age 19–24 years	No effect on body composition	Petridou *et al.* (2003)
6·0	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Safflower oil	8 weeks	Twenty-one subjects with type II diabetes	Levels of plasma *trans*-10,*cis*-12 CLA, but not *cis*-9,*trans*-11 CLA, were inversely associated with body weight	Belury *et al.* (2003)

1·8 and 3·6	Tonalin*	Oleic acid	13 weeks after 3 weeks on a very-low-energy diet	Fifty-four overweight males and females Age 37·8 years (mean)	No effect on percentage body weight regain. Increased regain of fat free-mass	Kamphuis *et al.* (2003*a*)
1·5 and 3·0	*Cis*-9,*trans*-11 *v.* *trans*-10,*cis*-12	High-oleic acid sunflower oil	18 weeks	Eighty-one healthy overweight males and females Age 35–65 years	No effect on weight, BMI or waist: hip ratio	Malpuech-Brugere *et al.* (2004)
4·5	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50 : 50, w/w)as NEFA or TAG	Olive oil	1 year	180 healthy overweight males and females Age 18–65 years	Decrease in body fat mass in both CLA groups Increase in lean body mass with CLA as NEFA, but not as TAG	Gaullier *et al.* (2004)
3·0	*Cis*-9,*trans*-11	Olive oil	12 weeks	Twenty-five abdominally-obese males Age 35–65 years	No effect on weight, BMI, body fat, lean body mass, SAD or waist circumference	Riserus *et al.* (2004*b*)
0·59, 1·19, 2·38 (*cis*-9,*trans*-11); 0·63, 1·26, 2·52 (*trans*-10,*cis*-12)	*Cis*-9,*trans*-11 *v.* *trans*-10,*cis*-12	None (cross-over design)	8 weeks on each dose (doses increased sequentially; 6-week washout between isomers)	Forty-nine healthy males Age 20–47 years	No effect on weight, BMI, fat mass or fat-free mass	Tricon *et al.* (2004*b*)

SAD, sagittal abdominal diameter; TAG, triacylglycerol.
*Composition (mg/g): 226, *trans*-10,*cis*-12, 236 *cis*-11,*trans*-13; 176, *cis*-9,*trans*-11; 166, *trans*-8,*cis*-10; 77, *trans*-9,*trans*-11 and *trans*-10,*trans*-12; 119, other CLA isomers.

hypothesis that CLA ingestion will lower the amount of accumulated body fat in adult subjects (Table 1). Only Kamphuis *et al.* (2003*a*,*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.* 2003*a*). 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.* 2003*a*). However, CLA was not found to affect percentage body-weight regain (Kamphuis *et al.* 2003*a*). Interestingly, measures of appetite (hunger, satiety and fullness) were also observed to be favourably and dose-dependently affected by CLA ingestion (Kamphuis *et al.* 2003*b*). 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.* 2002*b*; 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.* 2002*b*; 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.* (2002*b*), 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%; $P < 0{\cdot}01$, 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

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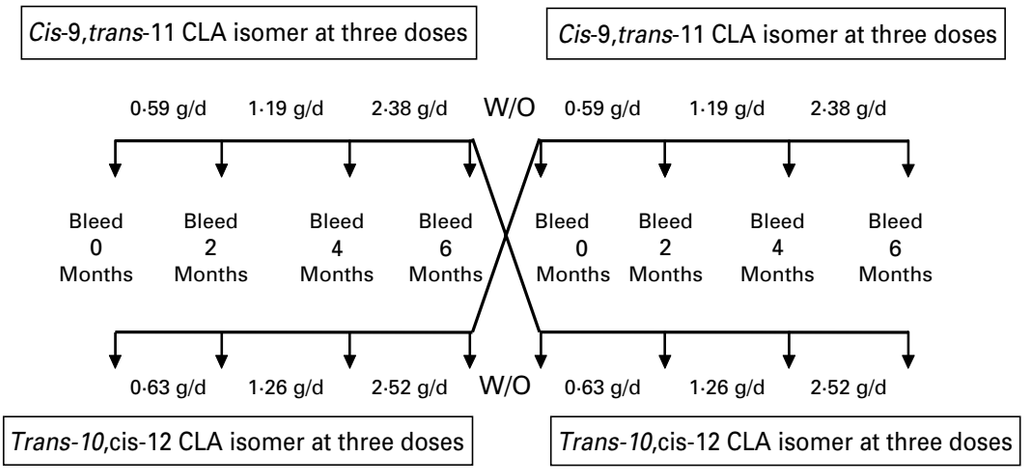

Fig. 1. Design of the study conducted by Burdge *et al.* (2004) and Tricon *et al.* (2004*a*,*b*). The subjects provided a fasting blood sample at baseline and then every 8 weeks during conjugated linoleic acid (CLA) supplementation. On each arm of the study the subjects consumed 0·59, 1·19 and 2·38 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.* 2004*b*; reproduced with permission from *American Journal of Clinical Nutrition*.)

profile. This possibility has been confirmed in a study investigating the specific effects of the two main CLA isomers at different doses on blood lipids in a double-blind randomized cross-over study in healthy normolipidaemic males (Tricon *et al.* 2004*b*). Dietary supplementation with highly-enriched *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA (0·59, 1·19 or 2·38 g/d and 0·63, 1·26 or 2·52 g/d respectively for 8 weeks) has been found to result in divergent effects of the two isomers on the blood lipid profile in healthy human subjects (Tricon *et al.* 2004*b*). Mean plasma triacylglycerol concentration, total cholesterol : HDL-cholesterol (Fig. 2) and LDL-cholesterol : HDL-cholesterol are increased after supplementation with the *trans*-10,*cis*-12 CLA isomer (Tricon *et al.* 2004*b*), demonstrating relative hyperlipidaemic properties of that isomer and hypolipidaemic properties of *cis*-9,*trans*-11 CLA. These results are in agreement with those of Riserus *et al.* (2002*b*), which suggest that the *trans*-10,*cis*-12 isomer may have some adverse effects on cardiovascular risk factors in obese subjects and in healthy subjects. This finding is of concern because the *trans*-10,*cis*-12 CLA isomer is found in equal proportions with the *cis*-9, *trans*-11 CLA in weight-loss products, which are available in health food stores and over the internet. However, the hypolipidaemic effects of the *cis*-9,*trans*-11 CLA observed by Tricon *et al.* (2004*b*) were not reported in the study of abdominally-obese men by Riserus *et al.* (2004*b*).

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.* 2002*b*, 2004*a*; Larsen *et al.* 2003). Riserus *et al.* (2002*b*) 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.* 2004*b*). They observed that *cis*-9,*trans*-11 CLA also decreases insulin sensitivity (by 15 %; $P < 0{\cdot}05$) compared with the placebo (Riserus *et al.* 2004*b*). 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.* 2002*b*). In another study (Fig. 1) Tricon *et al.* (2004*b*) 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,

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Table 2. Published studies investigating the effect of conjugated linoleic acid (CLA) on plasma lipids in human subjects

Amount of CLA (g/d)	Form of CLA	Placebo	Duration	Subjects	Effect of CLA	Reference
3·4	Tonalin*	Olive oil	12 weeks	Sixty overweight or obese males and females Age >18 years	No effect on total cholesterol, LDL- or HDL-cholesterol, or TAG	Berven *et al.* (2000)
1·7, 3·4, 5·1 and 6·8	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Olive oil	12 weeks	Sixty overweight or obese but healthy males and females	Decrease in total cholesterol, LDL- or HDL-cholesterol, or TAG	Blankson *et al.* (2000)
3·9	Tonalin*	Sunflower oil	9 weeks in a metabolic suite	Seventeen healthy females Age 20–41 years	No effect on total cholesterol, LDL- or HDL-cholesterol, or TAG	Benito *et al.* (2001)
0·7 then 1·4	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Soyabean oil	4 weeks then 4 weeks	Fourteen healthy males and ten healthy females Age 19–24 years	Decrease in HDL-cholesterol during low dose Tendency towards decrease in total cholesterol and TAG	Mougios *et al.* (2001)
4·2	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Olive oil	4 weeks	Twenty-four abdominally-obese males with signs of the metabolic syndrome Age 39–64 years	No effect on total cholesterol, LDL- or HDL-cholesterol, or TAG	Riserus *et al.* (2001)
4·2	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Olive oil	12 weeks	Twenty-seven healthy males and twenty-six healthy females Age 23–63 years	No effect on total cholesterol, LDL- or HDL-cholesterol, TAG or NEFA	Smedman & Vessby (2001)
3·0	*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)	Linoleic acid-rich vegetable oil	8 weeks	Eighteen healthy males and thirty-three healthy females Age 31·6 years (mean)	Decrease in TAG with the 50:50 (w/w) isomer mix Decrease in VLDL-cholesterol with the 80:20 (w/w) isomer mix	Noone *et al.* (2002)
3·4	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w) CLA *v.* *trans*-10,*cis*-12	Olive oil	12 weeks	Sixty abdominally-obese males with signs of the metabolic syndrome Age 35–65 years	Decrease in HDL-cholesterol with *trans*-10,*cis*-12 CLA Tendency to decreased HDL-cholesterol with the CLA mix	Riserus *et al.* (2002*b*)
2·1	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w)	Soyabean oil	45 d	Sixteen healthy females Age 19–24 years	No effect on total cholesterol, HDL-cholesterol, total cholesterol:HDL-cholesterol or TAG	Petridou *et al.* (2003)
4·5	*Cis*-9,*trans*-11 and *trans*-10,*cis*-12 (50:50, w/w) as NEFA or TAG	Olive oil	1 year	180 healthy overweight males and females Age 18–65 years	No effect on total cholesterol or TAG Decrease in HDL-cholesterol with CLA as TAG Increase in LDL-cholesterol with CLA as NEFA	Gaullier *et al.* (2004)
3·0	*cis*-9,*trans*-11	Olive oil	12 weeks	Twenty-five abdominally-obese males Age 35–65 years	No effect on total cholesterol, LDL-, HDL- or VLDL-cholesterol, or TAG	Riserus *et al.* (2004*b*)
0·59, 1·19, 2·38 (*cis*-9,*trans*-11); 0·63, 1·26, 2·52 (*trans*-10,*cis*-12)	*Cis*-9,*trans*-11 *v.* *trans*-10,*cis*-12	None (cross-over design)	8 weeks on each dose (doses increased sequentially; 6-week washout between isomers)	Forty-nine healthy males Age 20–47 years	Increased cholesterol:HDL-cholesterol, LDL-cholesterol:HDL-cholesterol and TAG with *trans*-10,*cis*-12 CLA relative to *cis*-9,*trans*-11 CLA	Tricon *et al.* (2004*b*)

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.

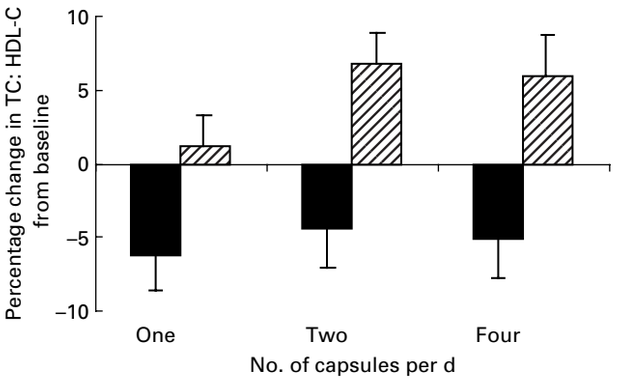

Fig. 2. Effects of *cis*-9,*trans*-11 conjugated linoleic acid (CLA; ■) and *trans*-10,*cis*-12 CLA (////) on the percentage change in total cholesterol:HDL-cholesterol (TC:HDL-C) from baseline. One, two or four capsules per d provided respectively 0·59, 1·19 or 2·38 g *cis*-9,*trans*-11 CLA/d or 0·63, 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.* 2004*b*; 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*. (2002*b*) 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*. 2004*b*).

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 amounts of several different isomers, in which *trans*-10, *cis*-12 and *cis*-9,*trans*-11 CLA isomers each represent about 200 mg/g) for 9 weeks (Kelley *et al*. 2000, 2001). None of the indices of immunity tested (number of circulating leucocytes, subsets within lymphocyte populations, T- and B-cell proliferation, delayed hypersensitivity skin response, and serum antibody titres after immunization with influenza vaccine) were reported to be affected by 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 E_2 and leukotriene B_4) 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 E_2) 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 (Burdge *et al*. 2004; Tricon *et al*. 2004*a*,*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 (Burdge *et al*. 2004) and on immune function (Tricon *et al*. 2004*a*; 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 (Burdge *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 (Burdge *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

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(Tricon *et al*. 2004*a*). 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 *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, which is inversely correlated with the proportions of both isomers in PBMC lipids (Tricon *et al*. 2004*a*). 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*. 2004*b*).

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.

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*. (2002*a*), 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 *trans*-10,*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 *trans*-10,*cis*-12-CLA-induced serum CRP elevation is not accompanied by an increase in IL-6 levels (Riserus *et al*. 2002*a*). In the study by Tricon *et al*. (2004*a*; Fig. 1), no marked effects on serum CRP concentration were found for either CLA isomer, suggesting that CLA supplementation with doses of ≤2·52 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 *trans*-10,*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.

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