Dietary conjugated linoleic acid and body composition

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Yanwen Wang and Peter JH Jones

ABSTRACT

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. The major dietary source of CLA for humans is ruminant meats, such as beef and lamb, and dairy products, such as milk and cheese. The major isomer of CLA in natural food is *cis*-9,*trans*-11 (*c*9,*t*11). The commercial preparations contain approximately equal amounts of *c*9,*t*11 and *trans*-10,*cis*-12 (*t*10,*c*12) isomers. Studies have shown that CLA, specifically the *t*10,*c*12-isomer, can reduce fat tissue deposition and body lipid content but appears to induce insulin resistance and fatty liver and spleen in various animals. A few human studies suggest that CLA supplementation has no effect on body weight and could reduce body fat to a much lesser extent than in animals. To draw conclusions on this form of dietary supplementation and to ultimately make appropriate recommendations, further human studies are required. The postulated antiobesity mechanisms of CLA include decreased energy and food intakes, decreased lipogenesis, and increased energy expenditure, lipolysis, and fat oxidation. This review addresses recent studies of the effects of CLA on lipid metabolism, fat deposition, and body composition in both animals and humans as well as the mechanisms surrounding these effects. *Am J Clin Nutr* 2004;79(suppl):1153S–8S.

KEY WORDS Conjugated linoleic acid, body weight, fat deposition, body composition, mechanisms, animals, humans

INTRODUCTION

Dietary conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. CLA is produced naturally in the rumen of ruminant animals by the fermentative bacteria, which isomerize linoleic acid into CLA. Ruminants also synthesize CLA by way of Δ^9-desaturase of *trans*-11-octadecanoic acid (1). The major dietary source of CLA for humans is ruminant meats, such as beef and lamb, and dairy products, such as milk and cheese (2, 3). The major isomer of CLA in natural foods is *cis*-9,*trans*-11 (*c*9,*t*11) (4, 5).

Research on the biological functions and health benefits of CLA dates back to the 1980s when Ha et al (6) made the seminal observation that CLA mixtures isolated from grilled beef, or from a base-catalyzed isomerization of linoleic acid, inhibited chemically induced skin neoplasia in mice. This discovery led to many studies that examined the beneficial effects of CLA on cancer, immune function, atherosclerosis, weight gain, and food and energy intakes, as well as body composition (7–29).

The antiobesity, antiatherogenic, and antidiabetic effects of CLA are supported by studies in animals (10–23), which led to the widespread use of CLA in the United States and Europe, especially among obese individuals. Commercial CLA supplements are isomeric mixtures, usually containing 2 major isomers, *c*9,*t*11 and *trans*-10,*cis*-12 (*t*10,*c*12), in equal amounts. There are several indications that various isoforms could have different biological actions, although most studies used a synthetically prepared CLA mixture. The *c*9,*t*11-isomer was implicated as the active form alone or in combination with other isomers responsible for the reported results of the mixed isomer preparations on tumorigenesis (5–7, 24–26). The *t*10,*c*12-isomer could be the active form that affects energy metabolism and body fat deposition and composition (11, 20). Apart from a possible antiobesity effect (27–29) in animals, the effects of CLA on the body weight and composition in humans are inconsistent and less significant than in animals. The objective of this review is to provide comprehensive information on the efficacy and mechanisms of antiadiposity of CLA in animals and humans.

CLA, BODY WEIGHT, AND BODY COMPOSITION IN ANIMALS

CLA decreases body weight in animals

The effect of CLA on body weight was investigated in various animal models, including mice, rats, and pigs (11, 12, 15, 16, 19, 22). Most animal studies have shown that CLA decreases weight gain (11, 12, 20, 22), whereas others have shown no effects (10, 16, 21, 23). The effect of CLA on body weight depends on the amount and isomer composition of the CLA mixture. When animals were supplemented with low amounts ($\leq$0.5% in the diet) of CLA mixture that contained approximately equal amounts of *t*10,*c*12- and *c*9,*t*11-isomers, the body weights were not affected (10, 21). However, when animals were given high amounts of CLA mixture that contained predominantly *c*9,*t*11-isomer, weight gain remained similar to control animals given safflower oil or low-CLA butter (11, 20).

Dietary supplementation of 1.5% (wt:wt) CLA, which contained 34% *t*10,*c*12-isomer and 33% *c*9,*t*11-isomer, reduced weight gain in male C57BL/6J mice and ICR mice (30). Similar results were reported in Zucker Diabetic Fatty (ZDF) rats (20) and ob/ob mice (31). In weanling female ICR mice, feeding

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0.25–0.50% CLA, containing 79% of the *t*10,*c*12-isomer, showed lowered body weights. No body weight reductions were observed when mice were supplemented with the same amounts of CLA mixtures that contained 44% or lower contents of *t*10,*c*12-isomer (11). These studies illustrate that the reduction in body weight gain after CLA supplementation is due to the action of the single isomer *t*10,*c*12-CLA.

One study demonstrated that greater reductions of weight gain by CLA were achieved in male AKR/J mice fed a high-fat (45% of calories) diet compared with the low-fat (15% of calories) diet (12). A dose-response effect of CLA was observed for weight gain in the aforementioned animal model when the animals were given a high-fat diet supplemented with 0.25–1.0% CLA (15). In a time-course study, it was found that in male AKR/J mice fed a high-fat diet (45% of calories), supplementation with 1.0% CLA reduced body weight after 22 d of CLA; the effect remained throughout a relatively long feeding period of 12 wk (15). As the study progressed, the difference between control and CLA treatment diminished, being virtually eliminated by week 12 (15). The noteworthy results of this study triggered the question of whether CLA can remain effective in reducing weight gain over the long term.

CLA reduces fat deposition in animals

Significant reductions in fat tissue mass were observed in animals fed diets supplemented with CLA. CLA supplementation at amounts as low as 0.5% reduced fat deposition in both male and female ICR mice (10). Marked reductions in both white and brown fat deposition were observed after feeding 1.0–1.5% CLA in female and male C57BL/6J mice (13, 19, 30). In a long-term study, female C57BL/6J mice were supplemented with 1.0% CLA for 5 mo, the results showed an ablation of brown adipose tissue and a marked decrease of subcutaneous white adipose tissue (19). In male Sprague-Dawley rats, the inclusion of 1.5% CLA for 3 wk reduced white adipose tissue weights (23). A significant reduction in the fat pad was also observed in ZDF rats when they were fed 1.5% CLA (20). In pigs, fat tissue deposition was reduced in a linear pattern as dietary CLA increased from 0.07% to 0.55% (16). In AKR/J male mice, 0.5–1.0% CLA supplementation resulted in marked reductions of fat tissue weights when they were maintained on a high-fat diet (45% of calories) (15). West et al (12) found that CLA reduced fat deposition equivalently in male AKR/J mice fed either a high-fat (45% of calories) or a low-fat (15% of calories) diet. There was no interaction between diet and CLA on adipose deposition (12). The supplementation of 0.5% CLA for 5 wk reduced fat pad weights in lean rats; in contrast, 0.5% CLA increased fat pad weight in obese rats (21), indicating an interaction between rat genotype and CLA. Results of these studies suggest that CLA supplementation reduces fat deposition in a dose-dependent manner with a dosage as low as 0.5% in the diet. It is also indicated that reductions of fat deposition by CLA are independent from dietary fat amounts but might depend on animal genotype.

Heterogeneous responses to CLA supplementation were observed in different adipose tissues. West et al (13) reported that in AKR/J male mice supplementation of 1.0% CLA reduced fat tissue weight mainly in the inguinal, epididymal, and the retroperitoneal regions, whereas the mesenteric fat deposition was unaffected. In the same animal model, the inclusion of 1.5% CLA within the diet resulted in a marked weight reduction in epididymal and perirenal white adipose tissues and interscapular brown adipose tissue. Parametrial white adipose tissue was shown to be less sensitive than other white adipose tissues (19). Other studies have shown that the retroperitoneal deposition was most sensitive to the effects of CLA and that the epididymal and mesenteric adipose depositions were relatively resistant to dietary CLA treatments (12, 15). In male ZDF rats, feeding 1.5% of CLA, containing approximately equal amounts of *t*10,*c*12- and *c*9,*t*11-isomers, showed significantly reduced weight gain, which was mirrored by smaller epididymal white fat pads and scapular brown fat pads, whereas *c*9,*t*11-isomer mixture (91% pure) had no effect on fat deposition (20). The effect of CLA on fat tissue deposition was shown to be attributable to *t*10,*c*12-isomer, instead of *c*9,*t*11-isomer and other isomers (11, 20).

CLA affects body lipid and protein contents in animals

In line with reports of CLA supplementation that effectively reduced fat deposition in animals, overall body lipid content was reduced, whereas the protein content increased (10, 11, 15, 22), except for one study in which protein content was decreased by CLA (12). The water and ash contents in animal carcass are also affected by CLA supplementation, with most studies showing increases in the body water and ash contents (10–12, 15, 16). The effect of CLA on body fat content is also independent of dietary fat amounts. When ARK/J mice were supplemented with 1.2% CLA in a high-fat (45% of calories) or 1.0% CLA in a low-fat (15% of calories) diet, body fat was reduced by both dietary treatments (12). In a dose-response test, DeLany et al (15) showed that in AKR/J mice body lipid content was reduced at the doses of 0.50%, 0.75%, or 1.0% of CLA in a high-fat (45% of calories) diet. An evident trend of increasing percentage of body protein with increases of dietary CLA reached significance at a dose of 1.0%, compared with controls, with carcass ash content unaffected (15). Similar results were observed in a 12-wk study in AKR/J mice (15). Results of other studies have shown that the body composition of animals can be altered by dietary *t*10,*c*12-isomer CLA (11, 20).

MECHANISMS OF CLA ON BODY WEIGHT AND BODY COMPOSITION IN ANIMALS

CLA alters energy balance

Although some studies have shown that CLA feeding reduces food or energy intake (11, 12, 15, 20, 30), the reductions are marginal and cannot fully account for the marked decreases in fat deposition. In addition, other reports have shown no effect on food or energy intake (10, 16, 22–23), even when large decreases in body fat mass were observed after CLA supplementation (10, 13, 21). Results of other studies suggest that fat mass reductions after CLA supplementation cannot fully be accounted for by decreases in food and energy intakes and other mechanisms must, therefore, be involved in CLA-induced fat reduction. West et al (12, 13) reported that CLA supplementation increased energy expenditure in AKR/J mice. Ohnuki et al (32) showed that CLA at an amount as low as 0.25% effectively increased energy expenditure and decreased white fat pad mass in male Std ddY mice. A recent study in Bald-C mice showed increased energy expenditure, excretion, and heat loss when animals were fed a

diet containing 0.93% CLA (22). It was also reported that the increased energy expenditure was sufficient to account for the decreased adipose deposition in CLA-treated mice (12).

Evidence suggests that uncoupling protein 2 (UCP2) plays an important role in CLA's regulation of energy expenditure. Substitution of CLA for 1% dietary fat increased UCP2 expression in brown adipose tissue in AKR/J male mice (13). Increased UCP2 mRNA amounts, caused by augmented CLA, were observed in parametrial white adipose tissue, the liver, or both of mice (19, 30, 31) and ZDF rats (20). Supplementation of CLA increased UCP2 mRNA amounts both in adipocytes and nonadipocytes, although a more pronounced effect was observed in adipocytes (19). Because UCP2 is a predominant uncoupling protein in white adipose tissue, up-regulation of UCP2 expression could contribute to the increased energy expenditure caused by CLA.

CLA inhibits lipogenesis

Animal studies have shown that CLA supplementation inhibits lipogenesis. For example, supplementation of 100 g/d of CLA mixture for 1 d decreased both fatty acid synthesis and fatty acid desaturation in cows (33). Other bovine studies showed that feeding 10 g/d of *t*10,*c*12-CLA decreased lipogenesis, while increasing plasma nonesterified fatty acid amounts (34, 35). Data supporting these findings were reported in other studies on the cultures of human preadipocytes (36–38) and stromal vascular cells from human adipose tissues (39).

One of the key enzymes in lipid metabolism is adipocyte lipoprotein lipase (LPL), which hydrolyzes fatty acids from circulating triacylglycerol, thus enhancing fatty acid uptake and re-esterification by the adipocytes. LPL activity in the cultures of fully differentiated 3T3-L1 adipocytes was reported to be reduced in a linear pattern by CLA supplementation, ranging from 20 to 200 μmol/L (10). Many in vitro studies using 3T3-L1 preadipocytes have shown that CLA decreases the concentrations of intracellular triacylglycerol and glycerol and increases the release of glycerol (10, 11, 40–44). The inhibitory effect of CLA on lipogenesis is correlated to inhibition of LPL activity (11, 40–42). The mRNA amounts of lipogenic enzymes, such as fatty acid synthase and acyl-CoA carboxylase, decreased markedly in female C57BL/6J mice after 5 mo of feeding with a diet containing 1% CLA (19). The expression of lipogenic enzymes is regulated by the transcription factor sterol regulatory element binding protein (45). The mRNA abundance of this protein showed a tendency to decrease with CLA feeding. Peroxisome proliferator-activated receptor-γ, another important transcription factor in adipogenesis, was also down-regulated in female and male C57BL/6J mice (19, 30) and in male ICR mice (30) after *t*10,*c*12-CLA isomer supplementation. These data collectively suggest that *t*10,*c*12-CLA decreases triacylglycerol content, in part, by decreasing fatty acid synthesis, uptake, and esterification into triacylglycerol by adipocytes, at least in certain species and model systems.

The inhibition of CLA on lipogenesis in adipocytes is an isomer-specific effect (11, 41). When 3T3-L1 adipocytes were incubated with 4 different CLA mixtures that contained 93% *t*10,*c*12, 44% *t*10,*c*12 and 41% *c*9,*t*11, 96% *c*9,*t*11, and 100% 9*t*11*t*, only the first 2 isomer mixtures showed a significant lowering of LPL activity and triacylglycerol concentration in the adipocytes (11). Dose-dependent reductions of LPL activity and intracellular triacylglycerol were observed in T3T-L1 adipocytes cultured with *t*10,*c*12-CLA in the medium (11). This inhibitory effect of *t*10,*c*12-CLA on LPL activity was confirmed by other studies (43, 44). In contrast, mixtures containing low amounts of *t*10,*c*12 and high amounts of *c*9,*t*11 produced no effect on intracellular triacylglycerol concentrations and LPL activity (11). Treatment of differentiating 3T3-L1 preadipocytes with *t*10,*c*12-CLA resulted in a dose-dependent decrease in the expression of stearoyl-CoA desaturase 1, an important enzyme in lipogenesis (41). In addition, cells treated with *t*10,*c*12-CLA isomer exhibited smaller lipid droplets and reduced amounts of the major monounsaturated fatty acids, palmitoleate and oleate. However, the *c*9,*t*11-isomer did not show any effect in altering the adipose gene expression or concentrations of palmitoleate and oleate (41). The results of these studies show that CLA has an inhibitory effect on adipogenesis.

CLA increases fat oxidation

Several studies demonstrated the ability of CLA to increase fatty acid oxidation. For instance, Sergiel et al (46) reported that radiolabeled *t*10,*c*12- and *c*9,*t*11-CLA were oxidized to a much greater extent than radiolabeled linoleic acid over a 2-h period in rats. Similarly, isolated perfused livers from rats fed 1% CLA mixture for 2 wk produced significantly more ketones compared with livers from 1% linoleic acid–fed rats (47). In addition, the ratio of β-hydroxybutyrate to acetoacetate increased, suggesting that dietary CLA exerts its hypolipidemic effect by increasing β-oxidation of fatty acids at the expense of fatty acid esterification. Indeed, an increased oxidation of [^{14}C]oleic acid was observed in 3T3-L1 preadipocytes treated with 50 μmol/L of *t*10,*c*12-CLA for 6 d (48). In agreement with this result, Martin et al (49) reported that *t*10,*c*12-CLA increased hepatic and adipose carnitine palmitoyltransferase activity, a rate-limiting enzyme for fatty acid β-oxidation, in rats that consumed a diet containing 1% *t*10,*c*12-CLA for 6 wk. Moreover, rats fed mixed CLA isomers had decreased respiratory quotients. These results, together with those of other studies (12, 32, 50), suggest that CLA increases fat oxidation rates.

CLA reduces adipocyte size

Reductions in fat cell size rather than fat cell number were indicated to be attributable to the decreases of fat deposition. In vitro studies showed significant decreases in cell size and accordingly decreases of triacylglycerol content in the adipocytes cultured in the presence of CLA (38, 51). This observation was verified by other studies in mice (19) and rats (21, 50, 52). The effects of the *t*10,*c*12-CLA isomer were more pronounced than the effects of a crude mixture of CLA isomers (51).

OBSERVED ADVERSE SIDE EFFECTS OF CLA IN ANIMALS

CLA induces insulin resistance

The possible beneficial effects of CLA supplementation in decreasing body fat mass have received a great deal of attention, but potential adverse effects of CLA on the insulin balance were largely ignored. In fact, hyperinsulinemia was observed in several animal studies in which animals were supplemented with CLA (15, 19, 31, 53, 54). CLA-induced insulin resistance could be related to the alterations of plasma leptin concentrations. Many studies have shown that CLA supplementation induced reductions of plasma leptin concentration in various animal mod-

els (15, 19, 23, 30, 55). A reverse correlation was observed between plasma *t*10,*c*12-isomer and leptin concentrations (55). Because leptin is an important hormone involved in maintaining blood glucose contestations by inducing insulin-mediated glucose disposal (56, 57), it is reasonable to consider that reductions of plasma leptin concentration by CLA affect insulin sensitivity. It was also reported that fatty acid–induced peroxidative stress is closely related to CLA-induced insulin resistance (54, 58). Results of those studies suggest that dietary CLA induces insulin resistance by reducing plasma leptin concentrations and increasing peroxidative stress. However, several studies did not show any effect of CLA on the blood glucose concentration (13, 15, 19, 30). Therefore, more studies are needed to examine any possible negative effect of CLA on insulin sensitivity and glycemic controls.

CLA induces fatty liver and spleen

Although CLA supplementation was shown to significantly reduce body fat and weight gain in different animal models, the concomitant enlargements of the liver and spleen have raised safety issues. In C57BL/6J mice, chronic supplementation with a 1% equimolar mixture of the *c*9,*t*11- and *t*10,*c*12-CLA isomers induced a marked loss of body fat but, meanwhile, caused massive fatty livers (19). Again, feeding 1.0% CLA in AKR/J mice for 6 wk increased the liver and spleen weights independent of body weight and dietary energy density (12, 13). In a dose-response study, CLA supplementation resulted in enlarged livers and spleens in male AKR/J mice, and this effect became significant at the dose of 1.0% CLA in the diet (15). Similar results were obtained in other studies in mice (59, 60). The tissue examination did not show any severe pathologic changes but increased lipid droplets in the liver and spleen (15, 19).

The biochemical, cellular, and molecular mechanisms involved in the development of fatty liver and spleen are not well established. It was suggested that fatty liver could be a consequence of the increased lipogenesis in the liver in compensating for the reduction of fat deposition in the adipose tissue (59, 61, 62). Evidence from several studies indicates that CLA-induced fatty liver is associated with hyperinsulinemia, which was discussed in detail by Clement et al (60).

CLA, HUMAN BODY WEIGHT, AND BODY COMPOSITION

The results of human clinical studies on the effects of CLA on body weight and composition are inconsistent (27, 55, 63–65). Reductions in body weight were only observed in patients with type 2 diabetes who received supplements of 6 g/d of CLA containing 39% *t*10,*c*12 and 37% *c*9,*t*11; a correlation was observed between body weight change and plasma concentrations of the *t*10,*c*12-isomer CLA (55). Other human studies did not show any effect of CLA (0.7–6.8 g/d) on body weight in healthy obese or nonobese men and women (29, 64, 65).

Reduction in body fat was observed in some human trials involving CLA supplementation (27–29, 65). An intensive CLA treatment (3.4 or 6.8 g/d for 2 wk) was found to be positively associated with a decrease in body fat mass in overweight and obese humans (27). In healthy nonobese subjects, the calculated percentages of body fat and fat mass were reduced by taking 1.4 g/d but not 0.7 g/d of CLA (51% *t*10,*c*12 and 49% *c*9,*t*11) for 4 wk (65). Thom et al (66) found that supplementation with 1.8 g/d of CLA for 12 wk reduced body fat in healthy exercising humans of normal body weight. Reductions of body fat proportion were also observed in healthy men and women who consumed 4.2 g/d of CLA for 12 wk, without any changes observed in body mass index and sagittal abdominal diameter (29). Riserus et al (28) found that abdominally obese men, who consumed 4.2 g/d of CLA for 4 wk, decreased sagittal abdominal diameter, without any concomitant effect on overall obesity. It should be noted that the reduction of fat mass by CLA supplementation is much less in humans than in animals and that there is no dose response of CLA observed about its effect on human fat mass (27, 29, 65, 66).

Animal studies have shown that reduced food and energy intakes and increased energy expenditure contribute to the reductions of body fat after CLA feeding (11, 13, 15, 20, 22). In humans, however, consumption of CLA did not show any effect on energy intake and expenditure, although body fat was reduced (55, 63–65, 67). Similarly, supplementation of CLA did not show any effect on fat oxidation and respiratory exchange rate in healthy women during resting or while walking (64). No effect on fatty acid or glycerol metabolism was observed in healthy, weight-stable, adult women given 3.9 g/d of CLA for 9 wk (68). However, CLA was shown to increase lipid peroxidation in middle-aged men with abdominal obesity (63) and healthy human subjects (67), indicating a potential adverse effect on cardiovascular system.

SUMMARY

Many studies in various animals and cell cultures have shown that CLA has the ability to reduce fat tissue deposition and lipid content in the body (10–16, 19–23). The inhibitory effect of CLA on adiposity is due to a single isomer CLA, *t*10,*c*12 (10, 11, 15, 16, 20, 22). The adiposity-lowering action of CLA also appears to be dose dependent and related to the duration of supplementation and species (12, 13, 15, 21). Supplementation with CLA induced fatty liver and insulin resistance in most animal studies (20, 31, 59, 60). Note that all animal experiments were conducted in young models, and the effects of CLA on the body weight and composition in mature animals remain unknown.

Most CLA supplementation studies are carried out for only a short duration (≈1–2 mo) of CLA supplementation (11, 12, 15, 20–22, 30). It has not been established whether CLA continues to alter adiposity in animals or humans over the long term. It is unclear whether consequences exist on termination of CLA supplementation. An increasing body of studies has tackled the mechanisms through which CLA regulates fat deposition (21, 23, 30, 36–38, 41, 43, 44, 50, 69). The postulated mechanisms mainly include decreasing energy intake and expenditure, decreasing lipogenesis, and increasing lipid oxidation. Thus far, only a few human studies have been conducted to examine the efficacy of CLA on altering the body weight and composition and to examine the mechanisms involved in this process, and the current results are inconsistent (27, 55, 64).

In conclusion, most of the evidence of CLA's effect on adiposity is based on animal studies. More studies are needed in humans to investigate the efficacy and mechanisms of specific CLA isomers as antiobesity agents. It is not clear whether there are any interactions between dietary CLA supplementation and energy intake, dietary fatty acid composition, especially polyunsaturated fatty acids, body mass index, and health conditions in humans. Additionally, the long-term effects and safety of CLA

need to be assessed. All of this knowledge is, however, critical for the development of CLA-fortified foods and CLA-based nutraceuticals for the prevention and treatment of nutrition-related obesity.

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