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REVIEW

Conjugated linoleic acid and obesity control: efficacy and mechanisms

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Obesity is associated with high blood cholesterol and high risk for developing diabetes and cardiovascular disease. Therefore, management of body weight and obesity are increasingly considered as an important approach to maintaining healthy cholesterol profiles and reducing cardiovascular risk. The present review addresses the effects of conjugated linoleic acid (CLA) on fat deposition, body weight and composition, safety, as well as mechanisms involved in animals and humans. Animal studies have shown promising effects of CLA on body weight and fat deposition. The majority of the animal studies have been conducted using CLA mixtures that contained approximately equal amounts of *trans*-10, *cis*-12 (t10c12) and *cis*-9, *trans*-11 (c9t11) isomers. Results of a few studies in mice fed CLA mixtures with different ratios of c9t11 and t10c12 isomers have indicated that the t10c12 isomer CLA may be the active form of CLA affecting weight gain and fat deposition. Inductions of leptin reduction and insulin resistance are the adverse effects of CLA observed in only mice. In pigs, the effects of CLA on weight gain and fat deposition are inconsistent, and no adverse effects of CLA have been reported. A number of human studies suggest that CLA supplementation has no effect on body weight and insulin sensitivity. Although it is suggested that the t10c12 CLA is the antiadipogenic isomer of CLA in humans, the effects of CLA on fat deposition are marginal and more equivocal as compared to results observed in animal studies. Mechanisms through which CLA reduces body weight and fat deposition remain to be fully understood. Proposed antiobesity mechanisms of CLA include decreased energy/food intake and increased energy expenditure, decreased preadipocyte differentiation and proliferation, decreased lipogenesis, and increased lipolysis and fat oxidation. In summary, CLA reduces weight gain and fat deposition in rodents, while produces less significant and inconsistent effects on body weight and composition in pigs and humans. New studies are required to examine isomer-specific effects and mechanisms of CLA in animals and humans using purified individual CLA isomers.
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Keywords: conjugated linoleic acid; body weight; fat deposition; mechanisms; animals; humans

Introduction

Conjugated linoleic acid (CLA) represents a group of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. CLA is naturally produced in the rumen of ruminant animals by fermentative bacteria, which isomerize linoleic acid into CLA. Ruminants also synthesize CLA via Δ9-desaturase of *trans*-11 octadecanoic acid.[1] The major dietary source of CLA for humans is ruminant meats, such as beef, lamb and dairy products including milk and cheese.[2,3] The major isomer of CLA in natural foods is *cis*-9, *trans*-11 (c9t11).[4,5]

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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 further studies examining the beneficial effects of CLA from different aspects including cancer, immune function, atherosclerosis, weight gain, food/energy intake, as well as body composition.[7–10]

The antiobesity effects of CLA have been supported by studies in animals.[10–24] Most of these studies used synthetically prepared CLA, a mixture of different isomers. Commercially prepared CLA supplements usually contain two major isomers, c9t11 and *trans*-10, *cis*-12 (t10c12), in equal amounts. The c9t11 isomer may be the active form, alone or in combination with other isomers, for the reported results of the mixed isomer preparations on tumorigenesis.[5–7,18,25,26] The t10c12 isomer may be the active form

affecting energy metabolism, weight gain and body fat deposition in animals, as indicated by the results of two studies in which mice were supplemented with CLA mixtures varying in the ratio of t10c12 and c9t11 isomers.[13,21] Despite the positive antiobesity effect of CLA in animals, the effects of CLA on body weight and composition in humans are inconsistent and less significant than those observed in animals.[27–29] Studies in humans[30] and human adipocytes[31] have demonstrated that t10c12 CLA is the antiadipogenic isomer of CLA. The objective of this review is to evaluate recent studies concerned with the effects of CLA on body weight and composition, fat deposition and safety, as well as mechanisms involved in animals and humans.

Effects of CLA on body weight and composition in animals

CLA reduces weight gain

Effects of CLA on body weight have been investigated in different rodent models (Table 1), including AKR/J mice, Balb-C mice, C57BL/6J mice, ICR mice, Sprague–Dawley rats, obese and lean Zucker rats.[11,13,15,20,23] Some studies have consistently shown reductions of weight gain by feeding CLA,[11,13,21,23] while others have not shown any effect.[10,12,22,26] Studies that have not shown a significant reduction in weight gain generally are those having applied either low levels ($\leq$0.5% in the diet) of CLA[11,22] or CLA mixtures that contained low concentrations of the t10c12 isomer.[13,21] Results of the studies conducted in pigs are controversial, with an increase,[32,33] decrease[34] or no effect[16,35,36] being observed.

It has been reported that supplementation with 1.5% CLA, containing 34% t10c12 and 33% c9t11, in a diet containing 13% added fat reduced growth rate in male C57BL/6J and ICR mice.[37] In weanling female ICR mice fed a diet with 6% added fat, diet supplementation with 0.25% of CLA, which contained a high level of the t10c12 isomer (79%), also produced a significant reduction in body weight. In contrast, no effect of CLA on body weight was observed when mice were fed the same diet supplemented with 0.5% CLA mixtures that contained 44% t10c12 and 42% c9t11 or 0.3% CLA containing 13% t10c12 and 72% of the c9t11 isomer.[13] The same group conducted another study and results showed that inclusion of 0.5% CLA mix containing 44% t10c12 and 42% c9t11 exhibited an inhibition on the weight gain in ICR mice, while supplementation with 0.5–0.9% CLA mix that contained 3% t10c12 and 29% c9t11 did not show any effect.[13] Similarly, supplementation with 1.5% CLA containing 48% t10c12 and 47% c9t11, in a diet with 5% added fat, reduced the weight gain in Zucker diabetic fatty (ZDF) rats.[21] Conversely feeding the same amount of CLA containing 91% c9t11, but only 1% t10c12 isomer, did not show any effect on the body weight.[21] Terpstra *et al*[23] found an interaction of CLA with feeding system on the weight gain, that is, a greater reduction of body weight was observed in the restricted than the nonrestricted male Balb-C mice fed with 0.9% of CLA (30% t10c12, 30% c9t11) for 39 days. Reductions of weight gain have also been reported in pigs supplemented with 0.5% CLA mixture containing 21% c9t11 and 16% t10c12 isomers.[34] Results of these studies suggest that CLA alters weight gain in growing animals when they are fed diets containing low or medium levels of fat (5–13%). It has also been indicated that the reduction of body

Table 1 Effects of CLA on body weight and energy intake in animals

Model	*Dietary fat (%)*	*CLA dose (% in diet)*	*CLA composition*	*Control*	*Duration (days)*	*Body weight*	*Energy/food intake*	*Reference*
AKR/J mice	29.2	1.2	41% t10c12, 39% c9t11	Corn oil	42	↓	↓	11
AKR/J mice	6.7	1.0	41% t10c12, 39% c9t11	Corn oil	42	↓	↓	11
AKR/J mice	5	0.25–1.0	41% t10c12, 39% c9t11	Corn oil	39	↓	↓	15
ICR mice	5.5	0.5	NA[a]	Corn oil	28–32	NS[b]	NS	10
Sprague–Dawley rats	4–10	1.5	47% t10c12, 46% c9t11	Safflower oil	24	NS	NS	24
Lean/obese Zucker rats	7	0.5	46% t10c12, 43% c9t11	Soybean oil	35–56	NS	NS	22
ICR mice	6	0.5	44% t10c12, 42% c9t11	Corn oil	28	NS	↓	13
ICR mice	6	0.25	79% t10c12, 16% c9t11	Corn oil	28	↓	↓	13
ICR mice	6	0.30	13% t10c12, 72% c9t11	Corn oil	28	NS	NS	13
Balb-C mice	20	0.93	30% t10c12, 30% c9t11	Sunflower oil	39	↓	NS	23
C57BL/6J mice	13	1.5	34% t10c12, 33% c9t11	Linoleic acid	21	↓	↓	37
ICR mice	13	1.5	34% t10c12, 33% c9t11	Linoleic acid	21	↓	NS	37
ZDF rats	5	1.5	1% t10c12, 91% c9t11	Low CLA butter	14	NS	↑	21
ZDF rats	5	1.5	48% t10c12, 47% c9t11	Low CLA butter	14	↓	↓	21
AKR/J mice	45	1	41% t10c12, 39% c9t11	Corn oil	35	NS	NS	12
Pigs		0.07–0.55	30% t10c12/c10t12, 25% c9t11/t9c11	Soybean oil	56	NS	NS	16
Pigs		0.12–1.0%	14% t10c12, 12% c9t11	Ground corn	26–114 kg	↑	NS	32
Pigs		2%	15% t10c12, 15% c9t11	Rapeseed oil	24–117 kg	NS	NS	35
Pigs		0.75%	20% t1012, 27% c9t11	Soybean oil	28–115 kg	NS	NS	36
Pigs		0.5%	NA	Soybean oil	38–73 kg	↓	NS	34

[a]Not available. [b]No significant difference as compared to control.

weight after CLA supplementation is likely due to the action of the single-isomer CLA, t10c12.

The effects of CLA in modulating weight gain have also been studied in mice fed high-fat diets. A reduction of body weight has been observed in *ob/ob* mice, fed a high-fat diet (30% kcal, as added fat), after feeding a 0.7% CLA mixture containing 82% t10c12 isomer, but not in those fed 0.7% CLA mixture containing 84% c9t11 isomer.[38] It was reported that supplementation of 1.0–1.2% CLA reduced weight gain in male AKR/J mice fed either low-fat (15% kcal, added as fat) or high-fat (45% kcal, added as fat) diets, with greater reductions observed in mice fed the high-fat diets.[11] A year later, the same research group examined the dose–response action of CLA on the weight gain in the same animal model given a high-fat diet (45% kcal, added as fat).[15] It was found that growth rate decreased over the CLA feeding period in a dose-dependent manner. A significant difference was observed after 18 days for 0.75% CLA and 21 days for 1% CLA groups, and both weights of groups remained lower thereafter until the end of the study. However, these observations were obtained over a relatively short feeding period of 39 days. In a time-course study, Delany *et al*[15] found that feeding male AKR/J mice with a high-fat diet (45% kcal, added as fat) containing 1.0% CLA reduced the body weight by day 22, and the effect remained throughout the relatively long feeding period of 12 weeks. As the study progressed, the difference between CLA treatment and control diminished, particularly by 12 weeks. Results of this study underscore the important question of whether CLA can remain effective in reducing weight gain of animals over a long-term period.

While most studies in mice have shown weight gain-lowering effect of CLA, a few studies have demonstrated no effect of CLA on body weight. Park *et al*[10] reported in both male and female ICR mice that inclusion of 0.5% CLA (isomer composition not defined) in the diet produced no effect on body weight. Similar results were observed in lean and obese Zucker rats supplemented with 0.5% CLA containing 46% t10c12 and 43% c9t11 isomers.[22] West *et al*[12] reported that AKR/J male mice fed a high-fat (45% kcal, as added fat) diet with 1% CLA containing 41% t10c12 and 39% c9t11 isomers did not show any difference in weight gain compared to those fed the high-fat diet alone. In another study in female C57BL/6J mice, feeding 1% CLA for a long period of 5 months did not affect body weight.[20] Other studies in mice and rats showed that CLA mixtures containing low concentrations of the t10c12 isomer had no effect on the growth rate.[13,21] In pigs, feeding 0.5% CLA containing 20% t10c12 and 27% c9t11 isomers reduced weight gain.[34] However, most pig studies have shown no change[16,35,36] or even increased weight gain[32,33] after supplementation with CLA mixture containing similar amounts of c9t11 and t10c12 isomers.

It is evident that the majority of the aforementioned animal studies have strongly demonstrated a weight gain-lowering effect of CLA mixtures, particularly those contained t10c12 isomer as one of the major components, in growing animals, although a few studies have failed to show an effect. The t10c12 isomer has been implicated to be the primary component of the CLA mixtures responsible for the body weight reduction. More studies are warranted to examine the isomer-specific effect of CLA on weight gain in animals.

CLA reduces fat deposition

Dietary CLA decreases adiposity in different animal models (Table 2), including mice,[10–13,15,20,23] rats,[21,22,24] hamsters[14] and pigs.[16,32,35,36,39] It has been reported that diet supplementation with CLA at a level as low as 0.5% reduced fat deposition in both male and female ICR mice.[10] Marked reductions in white and brown fat deposition have been observed in female and male C57BL/6J mice after feeding 1.0–1.5% CLA.[20,37] Decreased fat depositions were also observed in ICR and AKR/J mice when fed 1.0–1.5% CLA.[12,37] In a long-term study, female C57BL/6J mice showed an ablation of brown adipose tissue and marked decrease of subcutaneous white adipose tissue after supplemented with 1.0% CLA for 5 months.[20]

Reductions of fat deposition induced by dietary CLA have also been reported in rat models. In male Sprague–Dawley rats fed diets containing 4, 7 or 10% added fat, supplementation of 1.5% CLA for 3 weeks significantly reduced white adipose tissue weight.[24] Similar results have been obtained in female Sprague–Dawley rats supplemented with 0.25 or 0.5% CLA for 5 weeks.[40] A reduction in fat pad mass has also been observed in ZDF rats when they were fed 1.5% CLA.[21] Interestingly, results of a study showed that supplementation with 0.5% CLA for 5 weeks reduced fat pad weight in lean rats, but, in contrast, increased fat pad weight in obese rats,[22] indicating that CLA may interact with genotype to affect body weight in animals.

West *et al*[11] found that supplementation with 1.0–1.2% CLA reduced fat deposition equivalently in male AKR/J mice fed either a high-fat (45% kcal, added as fat) or a low-fat (15% kcal, added as fat) diet. No interaction was observed between dietary fat levels and CLA.[11] Another study showed that CLA supplementation resulted in marked reductions of fat tissue weights in male AKR/J mice fed high-fat (45% kcal, added as fat) diets.[15] Results of these studies show that CLA supplementation reduces fat deposition in mice regardless of dietary fat concentration.

In pigs, CLA supplementation produced controversial results.[16,32,34–36,39,41–43] It has been reported that back-fat thickness was significantly decreased in male and female pigs supplemented with 2.0% CLA mixture containing 15% c9t11 and 15% t10c12 isomers during the growing–finishing period (24–117 kg).[35] Similarly, Wiegnand *et al*[36] observed a decrease in fat deposition in growing–finishing (28–115 kg) pigs fed 0.75% CLA mixture containing 27% c9t11 and 20% t10c12 isomers. A linear pattern of reduction in the fat deposition was observed in finishing pigs when supplemented with 0.07 to 0.55% CLA containing 25% c9t11 and 31% t10c12 isomers.[16,39] O'Quinn *et al*[34] reported that fat

Table 2 Efficacy of CLA on adipose deposition and body composition in animals

Model	Dietary fat (%)	CLA dosage (% in diet)	CLA composition	Control	Duration (days)	Adipose deposition	Carcass lipid	Carcass lean/protein	Reference
AKR/J mice	29.2	1.2	41% t10c12, 39% c9t11	Corn oil	42	↓	↓	↓	11
AKR/J mice	6.7	1.0	41% t10c12, 39% c9t11	Corn oil	42	↓	↓	↓	11
AKR/J mice	5	0.25–1.0	41% t10c12, 39% c9t11	Corn oil	39	↓	↓	↑	15
AKR/J mice	5	1.0	41% t10c12, 39% c9t11	Corn oil	84	↓	↓	↑	15
ICR mice	5.5	0.5	NA[a]	Corn oil	28–32	↓	↓	↑	10
Sprague–Dawley rats	4–10	1.5	47% t10c12, 46% c9t11	Safflower oil	24	↓	NA	NA	24
Lean Zucker rats	7	0.5	46% t10c12, 43% c9t11	Soybean oil	35–56	↓	NA	NA	22
Obese Zucker rats	7	0.5	46% t10c12, 43% c9t11	Soybean oil	35–56	↑	NA	NA	22
ICR mice	6	0.5	44% t10c12, 42% c9t11	Corn oil	28	↓	↓	↑	13
ICR mice	6	0.3	13% t10c12, 72% c9t11	Corn oil	28	↓	↓	↑	13
ICR mice	6	0.25	79% t10c12, 16% c9t11	Corn oil	28	↓	↓	↑	13
ICR mice	6	0.5	3% t10c12, 29% c9t11	Corn oil	28	NS[b]	NS	NS	13
ICR mice	6	0.9	13% t10c12, 72% c9t11	Corn oil	28	↓	↓	↑	13
Balb-C mice	20	0.93	30% t10c12, 30% c9t11	Sunflower oil	39	↓	↓	↑	23
C57BL/6J mice	13	1.5	34% t10c12, 33% c9t11	Linoleic acid	21	↓	NA	NA	37
ICR mice	13	1.5	34% t10c12, 33% c9t11	Linoleic acid	21	↓	NA	NA	37
ZDF rats	5	1.5	1% t10c12, 91% c9t11	Low CLA butter	14	NS	NA	NA	21
ZDF rats	5	1.5	48% t10c12, 47% c9t11	Low CLA butter	14	NS	NA	NA	21
Pigs		0.07–0.55	30% t10c12/c10t12, 25% c9t11/t9c11	Soybean oil	56	↓	↑	NS	16
Pigs		0.07–0.55	30% t10c12/c10t12, 25% c9t11/t9c11	Soybean oil	56	↓	↓	↑	39
Pigs		0.75%	29% t10c12, 23% c9t11	Soybean oil	28–115 kg	↑	NA	↑	36
Pigs		2%	15% t10c12, 15% c9t11	Rapeseed oil	24–117 kg	↓	NA	↑	35
Pigs		0.12–1.0%	14% t10c12, 12% c9t11	Ground corn	26–114 kg	↓	NA	↑	32

[a]Not available. [b]No significant difference as compared to control.

deposition was unchanged in pigs given 0.5% CLA mixture (the composition of CLA was not provided) during the growing–finishing (38–73 kg) period. Another study also failed to show a significant effect of CLA mixture containing 21% c9t11 and 15% t10c12 isomers on fat deposition.[43] Conversely, increased fat depositions were reported in finishing pigs fed 1.0% CLA mixture containing 23% c9t11 and 29% t10c12 isomer,[41] or 1.0–5.0% CLA mixture that had the c9t11 and t10c12 isomers as two of the four major CLA isomers.[42]

Heterogeneous response to CLA supplementation has been observed in different adipose tissues. West *et al*[12] reported that in AKR/J male mice, CLA supplementation resulted in approximately 50% reductions of fat deposition in inguinal, epididymal or retroperitoneal region, while fat deposition in the mesenteric region remained unaffected. Another study showed that there was little renal, retroperitoneal and subcutaneous white adipose tissue or brown adipose tissue in CLA-fed mice.[20] The parametrial white adipose tissue was less sensitive to CLA than other white adipose tissues, but it was also reduced by 73% as compared to control mice.[20] Similarly, it has been shown that retroperitoneal adipose deposition is most sensitive and the epididymal and mesenteric adipose depositions were relatively resistant to dietary CLA supplementation.[11,15] A fat deposition-specific response of adipose tissues to CLA has also been reported in rats.[22,40]

The aforementioned studies have shown that diet supplementation with CLA containing approximately equal amounts of the c9t11 and t10c12 isomers inhibit fat deposition in adipose tissues. Park *et al*[13] reported that ICR mice did not show reductions in fat mass when they were supplemented with a CLA mixture that contained 3% t10c12 isomer, but significantly reduced the fat deposition when fed CLA mixtures containing 13, 44 and 79% of t10c12 isomer. Results of this study indicate that the t10c12 is the primary isomer responsible for the reductions of fat deposition induced by CLA mixtures. Again, as mentioned earlier, additional studies are needed to assess the isomer-specific effects of CLA on fat deposition by feeding animals with purified individual CLA isomers.

CLA affects whole body-composition

Being consistent with the reduction of fat deposition, overall body fat content has been shown to be reduced, while body protein content was increased after CLA supplementation,[10,13,15,23] with one exception in which protein content was decreased by CLA.[11] Water and ash contents of animal carcass are also affected by CLA supplementation.[10,11,13,15,16]

It has been reported that supplementation with 0.5% CLA in the diet reduced body fat content, but increased protein and water contents in both male and female ICR mice.[10] Supplementation of 0.25% CLA containing 79% t10c12 or 0.5% CLA containing 44% t10c12 and 41% c9t11 CLA isomers reduced body fat content and increased body protein, water and ash contents in weanling female ICR mice, whereas the CLA mixtures containing lower concentrations of the t10c12 isomer were less effective.[13] In weanling male ICR mice, diet supplementation with 0.5%

CLA that contained 44% t10c12 and 42% c9t11 showed a greater reduction in body fat content than feeding 0.5% and even 0.9% CLA that contained only 3% t10c12 but 29% c9t11 CLA isomers.[13] Significant reductions in body fat content were also observed in Balb-C mice when they were given a diet containing 0.93% CLA containing equal amounts (30%) of the t10c12 and c9t11 isomers.[22] When pigs were given a diet containing 0.07–0.55% CLA containing 30% t10c12/c10t12, 25% c9t11/t9c11 isomers, the carcass fat content was reduced, while water content was increased in a linear pattern with increasing CLA. The carcass lean tissue deposition response to CLA was quadratic and reached a plateau at 0.5% CLA in the diet. The ratio of fat to lean tissue deposition decreased linearly with the increase of dietary CLA.[16]

The effect of CLA on body composition in mice is independent of dietary fat levels. When ARK/J mice were supplemented with 1.2% CLA containing 41% t10c12 and 39% c9t11 isomers in a high-fat (45% kcal, added as fat) or 1.0% CLA in a low-fat (15% kcal, added as fat) diet, body fat was reduced by both dietary treatments.[11] In a dose–response study, Delany *et al*[15] found that body fat content of AKR/J mice was reduced at diet CLA doses of 0.50, 0.75 and 1.0% in a high-fat (45% kcal, added as fat) diet. There was an evident trend to increase the percent body protein with the increase of dietary CLA, and this trend became significant at the dose of 1.0% as compared with controls. The ash content of the carcass was not affected by CLA treatment. Similar results were observed in a 12-week time-course study in the same animal model.[15] Results of these studies demonstrated that body composition in animals can be altered by diet supplementation with CLA, and this effect is likely attributed to the t10c12 CLA isomer.

Mechanisms through which CLA affects weight gain and body composition in animals

CLA decreases energy intake and increases energy expenditure

Although many studies have demonstrated that dietary supplementation of CLA reduces weight gain, fat deposition and body fat content in growing animals, the antiadiposity mechanisms of CLA remain elusive. Some studies have shown that CLA supplementation reduces food/energy intake,[11,13,15,21,37] while others have not shown any effect[10,16,23,24,32,35,36] (Table 1). It was also reported that diet supplementation with CLA induced significant decreases in body fat deposition, but marginal reductions in energy intake.[11] Several studies have shown a large decrease in body fat mass after supplementation with CLA, even when there were no alterations in energy intake.[10,12,22] Data from these experiments strongly suggest that other mechanisms are involved in the observed CLA-induced declines of adipose deposition, while reductions in food intake may account in part for the reductions of weight gain and fat mass in animals.

On the other hand, West *et al*[11,12] observed a significant increase of energy expenditure in AKR/J mice after supplementation with 1% CLA in the diet. It was also reported that dietary CLA at a level as low as 0.25% effectively elevated energy expenditure and subsequently decreased white fat pad mass in male Std ddY mice.[44] A recent study in Balb-C mice showed higher energy expenditure, excretion and heat loss when animals were fed a diet containing 0.93% CLA.[23] West *et al*[12] compared the increase of energy expenditure with the reduction of fat deposition and found that the increased energy expenditure was sufficient to account for the decreased adipose deposition in CLA-treated mice.

It has been hypothesized that the enhanced thermogenesis in adipose tissues is partially attributed to the alterations in the expression of genes encoding uncoupling proteins (UCPs), a family of proteins that regulate adiposity and are expressed differently in various adipose and other tissues. While UCP1 is expressed exclusively in brown adipose tissue, UCP2 is expressed ubiquitously in multiple tissues, whereas UCP3 is expressed at high levels in skeletal muscle and brown adipose tissue.[45,46] West *et al*[12] failed to show any effect of CLA (1% in the diet) on UCP1 mRNA levels in adipose tissue and skeletal muscle of AKR/J male mice. A more recent study has however shown that supplementation with 1.5% CLA decreased UCP1 and UCP3 in brown adipose tissue in C57BL/6J and ICR male mice.[37]

In contrast, accumulating evidence suggests that UCP2 plays a more important role than UCP1 and UCP3 in the CLA-induced alterations of energy expenditure. West *et al*[12] reported that a substitution of 1% CLA for dietary fat increased UCP2 expression in brown adipose tissue, but not white adipose tissue, in male AKR/J mice. Increased UCP2 mRNA levels after feeding CLA have been observed in parametrial white adipose tissue and the liver of female C57BL/6J mice,[20,37] male ICR mice[37] and *ob/ob* mice.[38] Increased UCP2 gene expression by dietary CLA was observed in the white adipose tissues and skeletal muscle of ZDF rats and in the white adipose tissue of C57BL/6J mice.[21] Supplementation with CLA increased UCP2 mRNA levels in both adipocytes and nonadipocytes, with stronger effects observed in adipocytes.[20] Since UCP2 is a predominant UCP in white adipose tissue, upregulation of UCP2 is likely a primary mechanism through which CLA increases energy expenditure in animals.

CLA reduces fat cell size

An *in vitro* study demonstrated that postconfluent cultures of 3T3-L1 preadipocytes supplemented with CLA had less triglyceride and smaller cell size than cultures supplemented with similar amounts of linoleic acid.[47] Similar results have been reported by Brown *et al*[48] where postconfluent cultures of 3T3-L1 preadipocytes treated during the first 6 days of differentiation with the t10c12, instead of the c9t11 CLA isomer, decreased triglyceride content and accordingly reduced adipocyte size. The effects of the t10c12 isomer

were more pronounced than those of a crude mixture of CLA isomers.[47,48]

The decreased size rather than number of adipocytes contributes to the reductions of fat mass of adipose tissues after supplementation with CLA. This observation has been verified by *in vivo* animal studies. For instance, Tsuboyama-Kasaoka *et al*[20] reported that adipose tissues from female C57BL/6J mice fed 1% CLA had increased the number of small adipocytes and decreased the number of large adipocytes. In Sprague–Dawley rats, CLA reduced fat pad weight in retroperitoneal and parametrial adipose tissue sites due to decreased size rather than the number of adipocytes.[40,49] Feeding 0.5% CLA reduced fat pad weight due to the decreased size of adipocytes in male and female lean Zucker rats, but increased fat pad weight that went along with the CLA-induced increase in the size of adipocytes in male and female obese Zucker rats.[22] A similar phenomenon has been seen in a study conducted in female lean and obese Zucker rats.[22] These studies demonstrate that CLA-induced reduction of fat deposition is a result of the decreased adipocyte size.

CLA alters preadipocyte differentiation

Adipocyte differentiation is mediated by a series of programmed changes in gene expression.[50] The cascade of transcription factors, in particular, CAAT/enhancer-binding protein (C/EBP) and peroxisome proliferator-activated receptor (PPAR) families, controls the process of adipocyte differentiation.[51,52] PPARγ and C/EBPα play important roles in the differentiation of preadipocytes into adipocytes.[50,53] These transcription factors coordinate the expression of genes involved in creating and maintaining the adipocyte phenotype including the insulin-responsive glucose transporter 4 (GLUT-4), stearoyl-CoA desaturase (SCD) 1 and 2.[52] These genes are now being used as a model to study the mechanisms of cellular differentiation, tissue-specific gene expression and dietary regulation of gene expression.[54]

3T3-L1 preadipocytes are one of the few well-characterized model systems available to study cellular differentiation and proliferation of adipocytes *in vitro*.[52,54] Differentiation of 3T3-L1 preadipocytes has been shown to mimic faithfully the *in vivo* processes giving rise to cells that possess virtually all of the biochemical and morphological characteristics of adipocytes.[52] One possible target gene of CLA is C/EBPα, which is not expressed during proliferation,[55] but is highly induced by the onset of differentiation of 3T3-L1 preadipocytes.[56] Reduced expression of C/EBPα in 3T3-L1 preadipocytes after CLA supplementation has been observed.[57] Dietary CLA has also been shown to suppress the expression and activity of PPARγ.[30,57] Many studies have demonstrated that CLA possesses a capacity to inhibit the expression and activity of SCD[30,58,59] and GLUT-4.[20,37,57] Consequently, several studies have demonstrated that CLA inhibits differentiation and represses adipocyte gene expression during the *in vitro* differentiation of mouse 3T3-L1 preadipocytes into adipocytes.[47,60,61] Owing to the differentiation of preadipocytes into adipocytes is an essential step to get mature adipocytes, which possess capability to take up, synthesize and store lipids, the inhibition on the differentiation of preadipocytes by CLA attributes to the observed lower triglyceride content and small adipocyte size.

CLA increases apoptosis of adipocytes

Apoptosis is another important process that might be associated with the reductions of fat deposition and body lipid content induced by CLA supplementation. Tumor necrosis factor-α (TNF-α) is a cytokine that leads to apoptosis of adipocytes.[62] The exposure to TNF-α was shown to induce apoptosis in human adipose culture.[62] Feeding of CLA in C57BL/6J female mice resulted in increases of TNF-α mRNA level in white adipose tissue but decreases in skeletal muscle.[20] Marked increases of TNF-α level in the adipocytes of mice after feeding CLA were consistent with the increased apoptosis as measured by DNA fragmentation assay and DNA analysis.[20] TNF-α was shown to inhibit the synthesis of lipoprotein lipase (LPL),[63] acetyl-CoA-carboxylase (ACC) and fatty acid synthase (FAS).[64] In addition, TNF-α levels were found to be positively associated with UCP2 mRNA expression.[20] All these changes in enzyme activity and gene expression induced by the increased TNF-α in adipose tissue after feeding of CLA favor a decrease in fat cell size.

However, a positive relationship was observed between serum TNF-α concentration and fat deposition after CLA supplementation. Studies in male Sprague–Dawley rats[24] and mice[65] have shown decreases of serum TNF-α levels and fat deposition after the supplementation of 1.0–1.5% of CLA. Results of these studies suggest that CLA may affect differentially the concentrations of TNF-α in the serum and adipose tissue. More studies are required to examine the relationships between fat deposition and TNF-α concentration in different tissue sites after CLA supplementation.

CLA inhibits lipogenesis in the liver and adipose tissue

One of the effects of CLA that has been observed consistently is its ability to alter the fatty acid composition of tissues by reducing the levels of monounsaturated fatty acids.[66] Monounsaturated fatty acids, oleate and palmitoleate, are synthesized via the action of SCD from stearate and palmitate, respectively. Palmitoleate and oleate are the major monounsaturated fatty acids of membrane phospholipids and triglycerides found in differentiated 3T3-L1 adipocytes *in vitro* and mouse adipose tissue *in vivo*.[67] A proper ratio of saturated to unsaturated fatty acids is important in maintaining membrane fluidity; alteration of this ratio can influence a variety of physiological responses, including adiposity,[68] metabolic rate[69] and insulin sensitivity,[70] all of which are affected by CLA.

The CLA-induced reduction of fat deposition appears to be a result of decreased lipid accumulation of adipocytes. One

of the key enzymes in lipid metabolism is adipocyte LPL, which hydrolyzes the circulating triglyceride and release fatty acids, which are then taken and re-esterified by the adipocytes. Park *et al*[10] found that LPL activity in the cultures of fully differentiated 3T3-L1 adipocytes was reduced in a linear pattern by CLA ranging from 20 to 200 μmol/l. The inhibition of LPL activity is significantly correlated with the suppressing effect of CLA on lipogenesis.[13,58,59,71] The mRNA levels of lipogenic enzymes, FAS and ACC, showed marked decreases in female C57BL/6J mice after 5 months of supplementation with 1% CLA.[20] The expression of lipogenic enzymes is regulated by the transcription factor sterol regulatory element-binding protein (SREBP)-1.[72] SREBP-1 mRNA abundance showed a tendency to decrease with CLA feeding. PPARγ, another important transcription factor in adipogenesis, was also downregulated in female and male C57BL/6J mice[20,37] and male ICR mice[37] after supplementation of CLA mixture containing similar amounts of the c9t11 and t10c12 isomers. Many *in vitro* studies have shown that CLA causes lipid mobilization, resulting in decreased concentrations of intracellular triglyceride and glycerol and increased free glycerol in the culture medium of 3T3-L1 preadipocytes.[10,13,58,59,71,73,74] A most recent study has shown that t10c12, but not c9t11, CLA decreases the triglyceride content of human adipocytes in culture by decreasing glucose and fatty acid uptake.[30]

The inhibitory effect of CLA on the lipogenesis has been observed in cows and the cultures of human preadipocytes. In cows, feeding 10 g/day of the t10c12 CLA isomer decreased lipogenesis and increased plasma nonesterified fatty acid levels.[75,76] A similar study in cows has shown that supplementation with 100 g/day of CLA for 1 day decreased fatty acid synthesis and desaturation.[77] Supporting data have been reported by other studies using the cultures of human preadipocytes[48,78,79] and stromal vascular cells from human adipose tissue.[80] Data from the above studies collectively suggest that CLA decreases triglyceride content, in part, by decreasing fatty acid synthesis, uptake and esterification into triglyceride by adipocytes, at least in certain species and model systems.

Additional support for this hypothesis arises in the observation of shifts in GLUT-4 protein concentration, a rate-limiting step for glucose uptake in skeletal tissue, white adipose tissue and plasma membrane.[20,81] Supplementation with 1.0% CLA markedly downregulated GLUT-4 mRNA levels in white and brown adipose tissues, but upregulated GLUT-4 mRNA levels in skeletal muscle in mice.[20,37] Reductions of GLUT-4 mRNA level and protein concentration in adipose tissue by CLA indicate an inhibition of CLA on the conversion of glucose into fat.

The inhibition of CLA on lipogenesis in adipocytes exists as an isomer-specific effect.[13,58] When 3T3-L1 adipocytes were incubated with four different CLA isomer mixtures, which contained 93% t10c12, 44% t10c12, 96% c9t11 and 100% t9c11, respectively, only the first two mixtures showed significant effects on LPL activity and cellular triglyceride concentration.[13] Dose-dependent reductions of LPL activity and intracellular triglyceride concentration were observed in T3T-L1 adipocytes when they were incubated in the presence of the t10c12 CLA in the medium.[13] This observation has been supported by studies of Park and Pariza *et al*.[73,74] In contrast, mixtures containing low levels of the t10c12, but high levels of the c9t11, isomers produced no effect on the intracellular triglyceride levels and LPL activity.[13] Treatment of differentiating 3T3-L1 preadipocytes with the t10c12 isomer resulted in a dose-dependent decrease in the expression of the SCD1, another important enzyme in lipogenesis.[58] In addition, cells treated with the t10c12 isomer exhibited smaller lipid droplets and reduced levels of major monounsaturated fatty acids, palmitoleate and oleate. In contrast, the c9t11 isomer did not alter the gene expression in adipocytes and did not yield any effect on palmitoleate and oleate concentrations in adipose tissue.[58] Results of these studies indicate that suppression of lipogenesis by CLA is predominantly the t10c12 isomer-specific effect.

CLA increases fat oxidation in the liver and adipose tissues

Mitochondria and peroxisomes are subcellular organelles that consume oxygen and oxidize fatty acids. When oxidative phosphorylation in mitochondria is uncoupled or peroxisomes are induced, fatty acid oxidation increases without a proportional increase of ATP synthesis, resulting in energy waste as heat. The increase in fatty oxidation in peroxisomes may account for some of the reduction in adiposity seen with dietary CLA.[79]

Several studies have confirmed the ability of CLA to increase fatty acid oxidation. The t10c12 and c9t11 CLA were preferentially oxidized compared with linoleic acid over a 2-h period in rats.[82] Interestingly, CLA also induces an increase in fat oxidation. The isolated perfused livers from rats fed 1% CLA mixture for 2 weeks produced more ketones compared to those from 1% linoleic acid-fed rats.[83] In addition, the ratio of β-hydroxybutyrate: acetoacetate increased, suggesting that dietary CLA changes the status of reduction and oxidation in the cell, which may associate with the increased β-oxidation of fatty acids. Indeed, an increase was evident in the oxidation of ^{14}C-labeled oleic acid in 3T3-L1 preadipocytes treated with 50 μmol/l of the t10c12 CLA for 6 days.[84] In agreement with this study, Martin *et al*[85] reported that hepatic and adipose carnitine palmitoyltransferase (CPT) activity, a rate-limiting enzyme for fatty acid β-oxidation, was increased in rats after consuming a diet containing 1% of the t10c12 CLA for 6 weeks. Moreover, rats fed mixed CLA isomers produced lower respiratory quotients,[85] indicating increased oxidation in the body. These results, together with other studies,[11,40,44] suggest that CLA increases fat oxidation.

Acyl-CoA-oxidase (ACO), the rate-limiting enzyme in peroxisomal β-oxidation, is highly induced during proliferation of peroxisomes and is the biomarker most often used to assess peroxisome proliferation.[86] CYP4A1 is a lipid-

metabolizing enzyme involved in ω-hydroxylation of fatty acids allowing for increased rates of β-oxidation.[87] Fatty acid-binding protein (FABP) is a cytosolic lipid transport protein thought to be involved with shuttling fatty acids to and from the plasma membrane during phospholipid turnover.[88] Supplementation with 1.5% CLA in rats increased liver lipid content and ACO and FABP mRNA levels.[89] This observation has been supported by the finding that feeding 0.5–1.5% CLA increased ACO and FABP, as well as CYP4A1 levels in mice.[90] Supplementation of CLA in ICR and C57BL/6J mice increased the mRNA levels and activity of these three and many other enzymes involved in fat oxidation.[91] Induction of these fatty acid catabolic enzymes may explain how CLA modulates fat metabolism in the liver and adipose tissues.

Potential side effects of CLA supplementation 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 have been largely ignored. This is paradoxical because CLA-mediated hyperinsulinemia have been observed in several studies in mice.[15,20,38,92]

CLA-induced insulin resistance may be related to the alterations of plasma leptin levels. Studies have shown that CLA supplementation induced reductions of plasma leptin levels in various animal models.[15,20,24,37] Feeding male AKR/J mice with a high-fat (45% kcal, as added fat) diet containing 0.25–1.0% CLA reduced plasma leptin levels in a dose-dependent manner.[15] Similar results have been reported in fasting and feeding C57Bl/6J mice.[20,37] A study in male Otsuka Long–Evans Tokushima rats showed marked reductions of fat mass and serum leptin levels after dietary supplementation with 1.0% CLA.[93] Influence of CLA supplementation on fat deposition and serum leptin levels has also been examined in Sprague–Dawley rats, which were fed diets containing three different levels of fat (4, 7 and 10 % safflower oil).[24] Results showed that supplementation with 1.5% CLA reduced serum leptin levels at each of the three dietary fat levels. A reverse correlation was observed between plasma t10c12 isomer and leptin levels.[24] Correlations were also found between plasma leptin levels and white adipose tissue weights in the perirenal, epididymal and the combination of both sites. Similar effects have been observed in another study in the same animal model.[94] Results of these studies suggest that reductions in fat mass due to CLA (or reduced food intake) are associated with reductions in leptin, which induces insulin resistance.

Leptin is an important hormone involved in maintaining blood glucose levels by inducing insulin-mediated glucose disposal;[95,96] therefore, it is reasonable to consider that reductions of plasma leptin levels by CLA affect insulin sensitivity. In fact, several studies have shown increased blood insulin levels and/or insulin resistance.[15,20,38] Although there were no alterations of blood glucose concentration after oral glucose tolerance testing, insulin tolerance testing clearly showed a marked insulin resistance in CLA-fed female C57BL/6J mice.[20] In contrast, West *et al*[12] reported no effect of CLA supplementation for 5 weeks on plasma leptin and glucose levels in AKR/J male mice supplemented with 1% CLA in a high-fat (45% kcal, added as fat) diet. Other studies have shown that CLA supplementation did not produce any effect on plasma glucose levels in AKR/J mice,[15] or C57BL/6J or ICR mice.[37] It has been reported that fatty acid-induced peroxidative stress is closely related to CLA-induced insulin resistance.[31,97] Results of these studies suggest that dietary CLA induces insulin resistance by reducing plasma leptin and increasing peroxidative stress. However, the effect of CLA on the blood glucose concentration was inconsistent. 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 has been shown to reduce significantly 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 c9t11 and t10c12 CLA isomers induced a marked loss of body fat, but meanwhile caused massive fatty livers.[20] Again, feeding 1.0% CLA in AKR/J mice for 6 weeks increased the liver and spleen weights independent of body weight and dietary energy density.[11,12] 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 have been obtained in other studies in mice.[98,99] The tissue examination did not show any severe pathological changes but increased lipid droplets in the liver and spleen.[15,20]

The biochemical, cellular and molecular mechanisms involved in the development of fatty liver and spleen are not well established. It has been suggested that fatty liver could be a consequence of the increased lipogenesis in the liver in compensating the reduction of fat deposition in the adipose tissue. The effects of CLA on the liver fat may be partially controlled by PPARα,[89,100] as an evidence that CLA appears to be a potent activator of PPARα.[101,102] Indeed, both the c9t11 and t10c12 isomers have been shown to activate PPARα.[89] However, Peters *et al*[101] found that dietary CLA increased the mRNA levels of enzyme proteins relating to lipogenesis in the liver of wild-type and PPARα-null C57BL/6N mice, indicating that increased lipogenesis by CLA is not a result of increased PPARα. Takahashi *et al*[91] demonstrated that CLA, compared to linoleic acid or palmitic acid, up-regulated not only mRNA expression but also the activity of various enzymes involved in lipogenesis in ICR and C57BL/6J mice. A strong and specific induction of genes, such as

those encoding PPARγ, fatty acid translocase CD36 (FAT/CD36) and adipocyte lipid-binding protein (ALBP), normally expressed at only very low levels in the normal liver, has been observed in mice fed a diet enriched with the t10c12 CLA. Similar hepatic overexpression of the PPARγ gene has also been reported in fat-less A-ZIP/F-1 and aP2/DTA transgenic mice and in *ob/ob* mice, suggesting that it is a specific feature of fatty livers.[103] As FAT/CD36 and ALBP are cellular lipid-binding proteins, overproduction of these two proteins is likely to increase fatty acid uptake capacity in the liver. The increase in FAS mRNA levels in mice fed the t10c12-CLA demonstrates that the lipogenic activity of the liver is also specifically induced by this CLA isomer.[103]

Other transcription factors, such as liver-X-receptors (LXRs) and SREBP-1, play a critical role in hepatic lipid metabolism by controlling *de novo* fatty acid synthesis.[104,105] However, it has been demonstrated by other studies that CLA-induced fatty liver is not dependent on the specific activation/inhibition of PPARα,β/δ and γ.[99,101,106] It was recently suggested that the balance within the cell between oxysterols and polyunsaturated fatty acids, which interfere with LXR activation *in vitro*, is a crucial determinant of hepatic lipogenesis.[107] This effect may be accounted for by the concomitant induction of the SREBP-1a gene, which is known to be involved in the regulation of hepatic lipogenic program.[108]

It is paradoxical that fatty acid oxidation in the liver is also enhanced after CLA supplementation. Previous studies[90,101] indicated that CLA increased the mRNA levels of hepatic fatty acid oxidation enzymes. A recent study[91] showed that CLA increased the activity of various fatty acid oxidation enzymes accompanying the upregulation of mRNA expression of mitochondrial and peroxisomal fatty acid oxidation enzymes in ICR and C57BL/6J mice. In this study, CLA caused a marked increase of triglyceride in the liver despite the increase of hepatic fatty acid oxidation, indicating that the enhanced lipogenesis is primarily responsible for the hepatic triglyceride accumulation.[91] The increased mRNA levels and activity of enzymes involved in both fatty acid synthesis and oxidation in the mouse liver by dietary CLA suggest that hepatic fatty acid oxidation and synthesis are regulated in the same pattern by CLA. The enhanced gene expression and activity of lipogenic enzymes are possibly due to the large reduction in white and brown adipose pad mass. As white adipose tissue plays a crucial role in metabolizing and converting glucose to fatty acid for storage purposes, the large decrease caused by dietary CLA of white fat pad mass together with the downregulated GLUT-4 expression may result in a compensatory increase in hepatic lipogenesis to metabolize glucose. Indeed, evidence from several studies indicates that CLA-induced fatty liver is associated with hyperinsulinemia, which has been discussed in detail by Clement *et al*.[99]

A few studies have assessed the toxicity of CLA supplementation by measuring enzyme activity *in vivo* and *in vitro*. Cultures of murine 3T3-L1 preadipocytes in a growth medium with 0.5–10 mg/l of CLA did not show any cytotoxic effect on the proliferation and differentiation of 3T3-L1 preadipocytes, cell viability at 96 h postinoculation and 6 days postdifferentiation, as well as on the lactate dehydrogenase activity.[61] CLA has also been reported not to be cytotoxic to human breast cancer cells.[109,110] A recent study examined the *in vivo* toxicity of CLA by measuring serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in the liver of Sprague–Dawley rats.[23] Results showed no effect of CLA on serum GOT and GPT activities. More studies are warranted to examine the toxicity of dietary supplementation with CLA.

CLA and human body weight and composition

Human clinical studies conducted in the past few years have not shown consistent results in the effect of dietary CLA supplementation on body weight (Table 3). Most studies

Table 3 Effects of CLA on body weight and composition in humans

Subjects (F/M[a])	*Intervention period*	*Dosage (g/day)*	*CLA composition*	*Placebo*	*Body weight*	*Fat mass*	*Reference*
17 (0/17)	64 days	3.9	14.7% t10c12, 11.4% c9t11	Sunflower oil	NS[b]	NS	102
17 (0/17)	64 days	3.9	14.7% t10c12, 11.4% c9t11	Sunflower oil	NS	NS	108
10 (10/10)	12 weeks	1.8	50% t10c12, 50% c9t11	Hydrogel	NS	↓	104
53 (26/27)	12 weeks	4.2	50% t10c12, 50% c9t11	Olive oil	NS	↓	29
53 (26/27)	12 weeks	4.2	50% t10c12, 50% c9t11	Olive oil	NS	↓	106
24 (10/14)	8 weeks	0.7–1.4	51% t10c12, 49% c9t11	Soybean oil	NS	NS	103
52 (35/17)	12 weeks	1.7–6.8	50% t10c12, 50% c9t11	Olive oil	NS	↓	27
24 (0/24)	4 weeks	4.2	37% t10c12, 37% c9t11	Olive oil	NS	↓ sagital abdominal diameter	28
60 (0/60)	12 weeks	3.4	35% t10c12, 35% c9t11	Olive oil	NS	NS	31
60 (0/60)	12 weeks	3.4	35% t10c12, 35% c9t11	Olive oil	NS	NS	107
60 (NA)	12 weeks	3.4	NA[c]		NS	NS	109
23 (NA)	28 days	6	14.7% t10c12, 11.4% c9t11	Olive oil	NS	NS	110
51 (23/28)	8 weeks	3.0	50% t10c12, 50% c9t11	Linoleic acid	NS	NS	105
51 (23/28)	8 weeks	3.0	20% t10c12, 80% c9t11	Linoleic acid	NS	NS	105
12 (NA)	8 weeks	8	39% t10c12, 37% c9t11	Safflower oil	NS	NA	112
56 (26/28)	13 weeks	1.8–3.6	NA	Oleic acid	NS	NS	111

[a]Women to men ratio. [b]No difference compared to control. [c]Not available.

failed to show any reduction of body weight in healthy obese or nonobese men and women after supplemented with CLA ranging from 0.7 to 6.8 g/day.[27–29,111–120] Only a recent study conducted in type II diabetics showed that supplementation with 6 g/day of CLA (39% t10c12 and 37% c9t11) for 8 weeks produced an inverse correlation between plasma t10c12 levels and body weight changes.[121] These results suggested that CLA may reduce body weight of diabetes subjects, but not healthy obese and nonobese individuals. More studies are required to assess the effect of CLA on human body weight.

The effects of CLA on human body fat mass have been investigated on several occasions (Table 3). A few studies showed slight reductions of body fat mass.[27–29,112,113,115] An intensive CLA supplementation study (3.4 or 6.8 g CLA/day for 12 weeks) showed an inverse association of dietary CLA with body fat mass in overweight and obese individuals.[27] In another study[112] 22 healthy nonobese subjects were given 0.7 g/day of CLA for 4 weeks and then switched to 1.4 g CLA for another 4 weeks. Results showed that the calculated percentage of body fat and fat mass were reduced by taking 1.4 g/day but not 0.7 g/day of CLA. Thom *et al*[113] examined the efficacy of CLA on fat mass in healthy exercising humans with normal body weight. It was found that supplementation of 1.8 g/day of CLA for 12 weeks reduced body fat. Reductions of body fat mass were also observed in healthy men and women who consumed 4.2 g/day of CLA for 12 weeks, without any changes observed in body mass index and sagittal abdominal diameter.[29] Riserus *et al*[28] found that abdominally obese men consuming 4.2 g/day of CLA for 4 weeks had decreased sagittal abdominal diameter, without any concomitant effect on overall obesity. In contrast, other human clinical studies failed to show any effect of dietary supplementation of CLA on the fat mass.[31,111,116–120] It should be pointed out that reductions of body fat by CLA in these aforementioned human studies were much less than those observed in animals. In addition, there was no dose–response of CLA on human fat mass observed.[27,29,112,113] Human adipocytes synthesize very little fatty acids *de novo*. Rodents synthesize much more lipids *de novo* in adipose tissue than humans, and this may explain differential effects of CLA on lipogenesis between rodents and humans.

In humans, consumption of CLA did not show any effect on energy intake and energy expenditure, although non-significant trends in body fat were observed.[111,114,115,121,122] For example, Mougios *et al*[112] reported in healthy non-obese subjects that consumption of 0.7 or 1.4 g/day of CLA for 4 weeks did not affect energy intake. In another study, feeding healthy women with 3 g/day of CLA for 9 weeks did not produce any effect on energy intake.[111] In a recent study, supplementation with 6 g/day of CLA for 8 weeks in type II diabetes subjects failed to reveal any change of energy intake.[121] Similarly, CLA supplementation did not alter energy expenditure, fat oxidation or respiratory exchange rates in healthy women during rest or while walking.[102] Nor did supplementation of 3.9 g/day of CLA for 9 weeks impact fatty acid or glycerol metabolism in healthy, weight-stable, adult women.[111]

Results of *in vitro* studies have suggested that CLA possesses antioxidative properties.[7,123] However, recent results have shown considerable increase in biomarkers for enzymatic lipid peroxidation and nonenzymatic free radical induced lipid peroxidation after supplementation with CLA in middle-aged men with abdominal obesity[124] and healthy human subjects.[115] Supportive data have been obtained by Smedman *et al*,[125] where CLA supplementation induced lipid peroxidation via enzymatic and nonenzymatic pathways. Moreover, the CLA induced increases in enzymatic and nonenzymatic lipid peroxidation appeared to be dependent on the isomeral property of the CLA preparation.[125] Results of these studies indicated a potential adverse effect of CLA supplementation on the cardiovascular system.

Supplementation with CLA has shown inconsistent effects on the concentrations of leptin, insulin and glucose. It was reported that in healthy women, supplementation with 3 g/day of CLA for 64 days in healthy women produced a transitory decrease in leptin levels over the first 7 weeks and then returned to the baseline over the last 2 weeks. Insulin and glucose levels remained unaffected.[126] In type II diabetes subjects, CLA supplementation was associated with increases of fasting plasma glucose concentration; correlation analyses showed that plasma levels of the t10c12, but not the c9t11, CLA isomer was inversely associated with serum leptin.[121] Supplementation with t10c12 CLA caused isomer-specific insulin resistance in obese men with the metabolic syndrome.[116] In abdominally obese men, a consumption of 4.2 g/day of CLA for 4 weeks did not change plasma insulin and glucose levels.[28] Noone *et al*[114] reported that in healthy humans, feeding 3 g/day of CLA for 8 weeks did not alter plasma glucose and insulin levels. There were no changes observed in insulin and glucose levels after consumption of 4.2 g/day of CLA for 12 weeks in healthy humans.[29] Reductions of plasma leptin levels have also been observed in type II diabetics who were supplemented with 6 g/days of CLA for 8 weeks.[121]

It is worthy to point out that data from human studies concerning effects of CLA on the body weight and composition are limited. This lack of reliable data in humans necessitates further investigations before any conclusion can be drawn as to the possible clinical value and safety of CLA supplementation with a commercial CLA mixture as an approach to weight management.

Summary

Many studies in various animals and cell cultures have demonstrated that CLA possesses an ability to reduce fat tissue deposition as well as cellular and whole-body lipid content.[10–16,20–24,32,35,36] The inhibitory effect of CLA on adiposity has been shown to be likely due to a single-isomer CLA, the t10c12.[10,13,15,16,21,23,30,31] The antiadiposity action

of CLA also appears to be dependent on the dose of CLA, duration of supplementation, and species of animals.[10,13,15,22] In mice, supplementation of CLA reduces weight gain and far deposition independent of dietary fat levels.[11,15,38] Since all animal experiments were conducted in young growing individuals, effects of CLA on body weight and composition in mature animals remains unknown. Most of the animals studies have been carried out over periods of 1–2 months of CLA supplementation.[11,13,15,21–23,37] It has not been established whether CLA continues to offer antiadiposity action over the long-term period in animals or humans. Although no adverse effects of CLA have been observed in pigs,[32–36,39,41–43] fat liver and spleen as well as insulin resistance have been reported in mice after LCA supplementation.[11,12,15,20,38,98,99] Results of these studies have raised a question relevant to humans of whether it is safe to supplement with CLA. A large body of studies have tackled mechanisms through which CLA regulates fat deposition and body weight,[22,24,37,40,48,57,58,61,73,74,78,79] whereas antiobesity mechanisms of CLA still remains to be established. Possible mechanisms through which dietary CLA reduce body fat deposition has been summarized in Figure 1.

Human studies did not show a significant effect of CLA on body weight.[27–29,111–120] Some human studies showed slight reductions of body fat mass or calculated fat percentage after CLA supplementation,[27–29,112,113,115] while others failed to show any effect.[31,102,107–111] The effect of CLA on insulin sensitivity and glucose control are also inconsistent.[28,29,93,114,116,126]

In conclusion, most existing evidence of CLA efficacy on adiposity stems from animal studies, particularly those conducted in mice. The majority of these animal studies have examined CLA mixtures but not individual isomers for weight and fat-lowering properties. In humans, although it is indicated that t10c12 isomer is the antiadipogenic isomer, the effects of CLA on fat deposition are less significant and more equivocal as compared to results observed in animals. More experiments are needed in humans to ascertain the efficacy, and investigate mechanisms and safety of specific CLA isomers. In addition, a need exists to establish whether interactions exist between dietary CLA supplementation and factors such as energy intake, dietary fatty acid composition, especially polyunsaturated fatty acids, or body mass index in humans. Such information is critically important in ensuring the development of safe and efficacious foods fortified with CLA, or CLA-based nutraceuticals, for the prevention or treatment of diet-related obesity.

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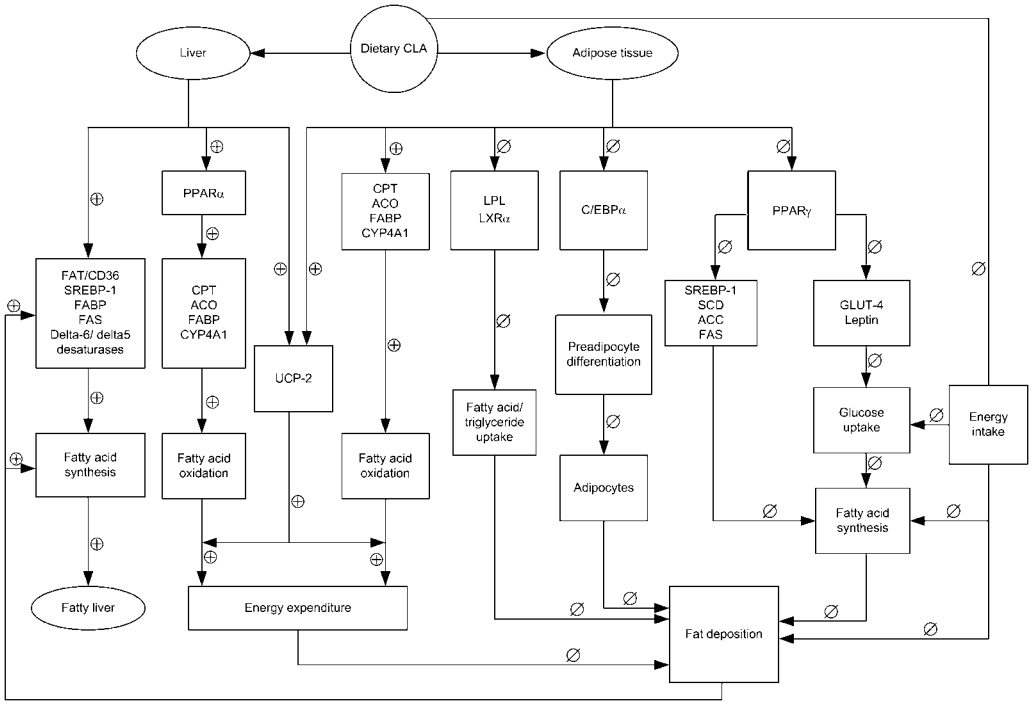

Figure 1 Possible mechanisms of antiadiposity action of conjugated linoleic acid. ⊕, enhance; ∅, suppress; ACC, acetyl CoA carboxylase; ACO, acyl-CoA oxidase; C/EBP, CCAAT/enhancer-binding protein; CPT, carnitine palmitoyltransferase; CYP4A1, cytochrome *P*450IV A1; FABP, fatty acid-binding protein; FAT/CD36, fatty acid translocase CD36; FAS, fatty acid synthase; GLUT, glucose transporter; LPL, lipoprotein lipase; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element binding protein; UCP, uncoupling protein.

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