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Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism

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Abstract

Isomers of conjugated linoleic acid (CLA), unsaturated fatty acids found in ruminant meats and dairy products, have been shown to reduce adiposity and alter lipid metabolism in animal, human, and cell culture studies. In particular, dietary CLA decreases body fat and increases lean body mass in certain rodents, chickens, and pigs, depending on the isomer, dose, and duration of treatment. However, the effects of CLA on human adiposity are conflicting because these studies have used different mixtures and levels of CLA isomers and diverse subject populations. Potential antiobesity mechanisms of CLA include decreased preadipocyte proliferation and differentiation into mature adipocytes, decreased fatty acid and triglyceride synthesis, and increased energy expenditure, lipolysis, and fatty acid oxidation. This review will address the current research on CLA's effects on human and animal adiposity and lipid metabolism as well as potential mechanism(s) responsible for CLA's antiobesity properties.

Keywords: Conjugated linoleic acid (CLA); Adiposity; Lipid metabolism; Antiobesity; Preadipocytes; Adipocytes

1. Introduction

What is CLA and where is it found? A naturally occurring fatty acid (see Fig. 1 for structure), CLA is produced in the rumen of ruminant animals by the fermentative bacteria, *Butyrovibrio fibrisolvens*, which isomerizes linoleic acid into CLA. Another source of CLA in ruminants is synthesis via Δ 9-desaturase of trans-11 octadecanoic acid [1]. *In vivo*, CLA is found in dairy products such as milk and cheese as well as ruminant meats such as beef and lamb (Table 1). In cheese, the amount of CLA ranges from 3.6 to 8.0 mg/g lipid while milk products contain 3.4 to 6.4 mg/g lipid [2]. Depending on the animal species, tissue, and diet, CLA content in ruminant meat products varies from 2.7 to 5.6 mg/g lipid [3]. Finally, butter contains approximately 720 mg/100 g food (wet weight) [4]. The relative quantities of CLA isomers in commercially available crude mixtures (i.e., ~41% cis-9, trans-11, 44% trans-10, cis-12, 7% trans-9, trans-12 CLA) and food (i.e., ruminant meats and milk contain ~80% cis-9, trans-11 CLA and 10% trans-10, cis-12 CLA) varies considerably [5]. Serum levels of CLA isomers in non-vegetarians have been reported to be in the 20–70 μM range, with cis-9, trans-11 and trans-10, cis-12 isomers representing 80 and 10%, respectively [5].

The first hint that CLA may be important. In 1987, Michael Pariza's group at the University of Wisconsin at Madison made the seminal observation that CLA, mixtures of geometric and positional conjugated dieonic isomers of linoleic acid isolated from grilled beef, or from a base-catalyzed isomerization of linoleic acid, inhibited chemically-induced skin neoplasia in mice [6]. This discovery led their group to a flurry of research examining CLA's beneficial effects on cancer [7], immune function [8], atherosclerosis [9], weight gain and food intake [10], and body composition [11]. On the basis of these studies, it was concluded that feeding (0.5%, w/w) a commercially available crude mixture of CLA isomers prevented chemically-induced tumors, protected against the catabolic effects of immune stimulation, improved feed efficiency, reduced excess weight gain, reduced body fat, increased lean body mass, and lowered blood lipids. Other studies support CLA's anticarcinogenic (for review, see 12, 13), antiatherogenic [9,14], antidiabetic [15], as well as antiobesity [11,15–25] properties.

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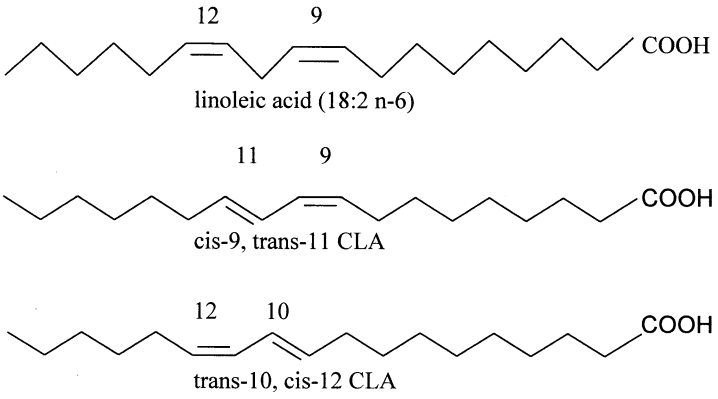

Fig. 1. Structure of linoleic acid, cis-9, trans-11 CLA and trans-10, cis-12 CLA.

2. CLA decreases body fat and increases lean body mass

Dietary CLA decreases adiposity in various animal models and in humans. For example, rodents fed 1–1.5% CLA (w/w) as a crude mixture of cis-9, trans-11 and trans-10, cis-12 isomers had less body fat and greater lean body mass than control animals [11,15–17,20–23]. Feeding 0.05–1.0% (w/w) mixed CLA isomers to pigs also increased feed efficiency and reduced back fat without affecting total body weight [24]. Similarly, feeding 0.07–0.5% (w/w) mixed CLA isomers to growing pigs for 8 weeks increased feed efficiency and lean body mass while reducing fat deposition compared to control pigs [25].

Feeding Sprague-Dawley rats 0.25–0.5% (w/w) of a crude mixture of CLA isomers for 5 weeks reduced retroperitoneal and parametrial fat pad weights without affecting growth rate or food intake [21]. Moreover, these reductions in fat pad weights were attributable to a decrease in adipocyte size rather than cell number. In contrast, Tsuboyama-Kasaoka et al. (2000) found that adipocytes isolated from female C57BL/6J mice underwent apoptosis following 4–28 days supplementation with 1% (w/w) mixed CLA isomers [20]. Tumor necrosis factor-alpha (TNF-α), a known inducer of apoptosis in adipocytes [26], levels also increased in mice fed the mixed CLA isomer diet. However, Yamasaki et al. [27] found that Sprague-Dawley rats fed 1–2% (w/w) mixed CLA isomers had no difference in body weight, food intake, or feed efficiency compared to the controls, although the CLA-treated animals did have lower levels of triglyceride (TG) in white adipose tissue, liver, and serum. Furthermore, the levels of non-esterified fatty acids in each of these tissues were decreased.

Table 1
CLA content of various foods*

Food	mg/g fat	Food	mg/g fat
Dairy product		**Meats/fish**	
Condensed milk	7.0	Lamb	5.8
Colby	6.1	Fresh ground beef	4.3
Butter fat	6.1	Veal	2.7
Ricotta	5.6	Fresh ground turkey	2.6
Homogenized milk	5.5	Chicken	0.9
Cultured buttermilk	5.4	Pork	0.6
American processed cheese	5.0	Pork	0.6
Mozarella	4.9	Egg yolk	0.6
Plain yogurt	4.8	Salmon	0.3
Custard style yogurt	4.8		
Butter	4.7		
Sour cream	4.6	**Vegetable oils**	
Cottage	4.5		
Low fat yogurt	4.4	Safflower oil	0.7
2% milk	4.1	Sunflower oil	0.4
Mediam cheddar	4.1	Peanut	0.2
Ice cream	3.6	Olive	0.0
Parmesan	3.0		
Frozen yogurt	2.8		

* Based on values reported by Lin et al. (2), Chin et al. (3), & the University of Wisconsin Food Research Institute (Dr. Pariza, Director).

These data suggest that dietary CLA reduces adiposity in several animal models, thereby inhibiting the development of obesity-related diseases. However, pigs fed 1% (w/w) mixed CLA isomers for 8 weeks as part of an isoenergetic diet showed no change in heat production, energy retention, or body weight [28]. Secondly, obese Sprague-Dawley rats fed 2% (w/w) mixed CLA isomers plus a high fat diet for 8 weeks following a 9 week period of food restriction exhibited no differences in fat regain, plasma leptin, or insulin levels compared to controls, although lipoprotein lipase (LPL) activity was increased [29]. Thus, experimental conditions such as age, type, and metabolic status of the animal model, as well as the level, isomer, and duration of CLA treatment may play an integral role in how CLA affects body composition. One proposed mechanism for CLA's antiobesity action is that it increases metabolic rate, thereby increasing energy expenditure [16]. For example, two studies in AKR/J mice found that supplementation with 1% (w/w) mixed CLA isomers increased energy expenditure without upregulating uncoupling protein (UCP) gene expression in skeletal muscle, white adipose tissue, or kidney, although UCP gene expression was slightly increased in brown adipose tissue [22,23].

Although the work cited above provides convincing evidence that CLA's antiobesity actions in animals are isomer- and dose-specific, *the physiological relevance and interpre-*

tation of these data for the prevention or treatment of human obesity are less clear and conflicting. For example, CLA treatment (3.4−6.8 g/day) for 6 months reduced body fat mass of obese and overweight adult men and women [30]. Similarly, CLA supplementation (4.2 g/day, mixed isomers) of middle aged adults for 3 months decreased the proportion of body fat compared to controls [31]. Exercising adults consuming 1.8 g/day mixed CLA isomers for 12 weeks had reduced body fat compared to placebo controls [32]. In contrast, CLA supplementation (3–3.4 g/day, mixed isomers) over 2–3 months did not affect fat mass, fat-free mass, percent body fat, body weight, or blood lipids of adults [33–36]. This discrepancy may be due to the type and amount of CLA isomers consumed, treatment duration, and body weights and energy intakes of the subjects. For example, many rodent studies found that feeding 0.5% CLA (mixed isomers) in the diet (w/w) for 6−8 weeks reduced adiposity. Assuming a 300 g growing rat consumes 15 g of feed daily, this would equal ~75 mg CLA/day or 225 mg/kg body weight. In contrast, several human studies have fed only 3 g/day of CLA (mixed isomers) for 6–12 weeks. For a 70 kg adult, this is ~ 43 mg/kg body weight of CLA and ~5 times less than the 0.5% (w/w) level fed to rodents. Therefore, the dose for a 70 kg person that would be equivalent to the dose used in rodent studies would be 16 g/day of CLA (mixed isomers). *Currently, the effect of trans-10, cis-12 CLA on human adiposity is unknown.*

Another question concerning CLA studies is which isomer(s) of CLA is responsible for its antiobesity effects. When hamsters were fed a hypercholesterolemic diet supplemented with either 1% (w/w) mixed CLA isomers, 0.2% (w/w) cis-9, trans-11 CLA, or 1% (w/w) linoleic acid, animals consuming the mixed CLA isomer diet had significantly lower weight gain, but greater food intakes than animals consuming either the cis-9, trans-11 CLA or linoleic acid diet [37]. Another study suggested that the trans-10, cis-12 isomer is the more potent antiobesity agent in mice compared to other CLA isomers, although the direct effects of the isomers were not determined in these animals [19]. This led Pariza's group to the discovery that feeding CLA enriched in the trans-10, cis-12 isomer decreased body fat of mice to a greater extent than a mixture enriched in cis-9, trans-11 CLA [18]. Although these data suggest that the trans-10, cis-12 isomer of CLA is the more potent antiobesity isomer in animals, more research is needed using individual CLA isomers in humans.

3. CLA isomers differentially affect adipogenesis

CLA has been shown to inhibit the growth and differentiation of preadipocytes into mature, TG-rich adipocytes in culture. For example, Park et al. [11] demonstrated that cultures of mature murine 3T3-L1 adipocytes treated with 20–200 μM of crude mixture of CLA isomers for 2 days had 8% less lipid, 66% lower LPL activity, and 22% more glycerol release (e.g., lipolysis) compared to control cultures. Subsequently, Park et al. [19] reported that the cultures of mature 3T3-L1 adipocytes treated with 44 μM trans-10, cis-12 isomer of CLA or 100 μM of a crude mixture of CLA isomers had 66% less LPL activity, 55% less TG content, and 1.8-fold more lipolysis than control cultures. In contrast, treating cultures with cis-9, trans-11 or trans-9, trans-11 isomers had no impact on cellular lipid status. However, these authors did not examine CLA's influence on preadipocyte proliferation or differentiation.

Satory and Smith [38] found that low levels (1−6 μM) of a crude mixture of CLA isomers reduced the proliferation of 3T3-L1 (pre)adipocytes, but increased their rate of differentiation (i.e., stimulated lipid accumulation). Contrary to these data, Brodie et al. [39] found that 25–100 μM of a crude mixture of CLA isomers inhibited the proliferation of cultures of 3T3-L1 preadipocytes. Furthermore, mRNA levels of two critical transcription factors, peroxisome proliferator-activator receptor gamma (PPARγ) and CCATT enhancer binding protein alpha (C/EBPα), and adipose fatty acid binding protein (aP2) were lower in CLA-treated cultures compared to control cultures, suggesting that CLA attenuated (pre)adipocyte differentiation.

Our group [40] and Pariza's group [18] have demonstrated that mixed isomers of CLA, and more specifically the trans-10, cis-12 isomer of CLA, reduce TG content in differentiating 3T3-L1 preadipocytes. Furthermore, these effects were shown to be dependent on treatment period, as TG content in cultures treated with trans-10, cis-12 CLA throughout the entire differentiation period was decreased to a greater extent than in cultures treated only during either the first 3 days or last 3 days of differentiation [41]. Evans et al. [40,41] also demonstrated that the TG-lowering effects of mixed CLA isomers and the trans-10, cis-12 isomer of CLA were significant even in the presence of exogenous antioxidants, suggesting lipid peroxidation did not play a role in CLA's TG-lowering actions. Finally, concurrent supplementation of 3T3-L1 preadipocytes with increasing doses of linoleic acid resulted in a dose-dependent increase in TG content compared to cultures treated with trans-10, cis 12 CLA alone, suggesting CLA's suppression of TG accumulation is reversible [41]. Linoleic acid supplementation also prevented some of the morphological changes associated with trans-10, cis-12 CLA treatment as seen with scanning electron microscopy [41]. This rescuing effect of linoleic acid has also been shown in cultures of human hepatocytes [42] and adipocytes [43,44] treated with CLA.

In examining CLA's effects on adipocyte differentiation and gene expression, Choi et al. [45] determined that 10–100 μM trans-10, cis-12 CLA reduced stearoyl-CoA desaturase –1 (SCD-1) gene expression without affecting PPARγ2, C/EBPα, aP2, SCD-2, or fatty acid synthase gene expression in 3T3-L1 adipocytes. SCD-1 is responsible for the desaturation of saturated fatty acids (SFA) such as palmitate (16:0) and stearic acid (18:0) into monounsaturated fatty acids (MUFA), including palmitoleic acid (16:1)

and oleic acid (18:1) necessary for synthesis and storage of TG. Brown et al. [46] similarly found that although neither acute or chronic treatment of differentiating human (pre)adipocytes with trans-10, cis-12 or cis-9, trans-11 CLA influence the protein levels of PPARγ or C/EBPα, the trans-10, cis-12 isomer increased the SFA:MUFA ratio. This suggests that trans-10, cis-12 CLA selectively decreases the synthesis of MUFA, thereby increasing the ratio of SFA to MUFA without inhibiting adipocyte differentiation per se.

In contrast, Ding et al. [47] found that isolated mature porcine adipocytes treated with 50–300 μM cis-9, trans-11 CLA for 24 h exhibited increased Oil Red O (ORO) staining compared to trans-10, cis-12 CLA, or linoleic acid. However, neither isomer altered PPARγ mRNA levels indicating that although CLA may act as a ligand for PPARγ, it does not induce PPARγ expression. Finally, Evans et al. [41] showed that PPARγ2 protein level increased following acute (2 days) treatment with trans-10, cis-12 CLA or cis-9, trans-11 CLA. However, PPARγ2 protein level decreased following chronic (6 days) treatment with trans-10, cis-12, but not cis-9, trans-11, CLA treatment. Thus, CLA may have different effects on adipocyte differentiation depending on isomer type and concentration, treatment period, culture conditions, and species. Therefore, more research is needed to determine exactly how CLA inhibits TG accumulation and more specifically, if it affects preadipocyte differentiation versus lipid metabolism.

4. CLA Alters Lipid Metabolism In vivo and In vitro

Insulin sensitivity and lipogenesis. CLA has been reported to alter glucose and lipid metabolism in animal and cell culture models. A study in Zucker fatty rats, a model for Type 2 diabetes, found that 1.5% (w/w) dietary CLA (mixed isomers) improved insulin sensitivity, glucose tolerance, hyperinsulinemia, and hyperglycemia after 14 days of treatment [15]. In contrast, consuming mixed isomers of CLA induced a tissue-specific insulin resistance in adipocytes of non-obese mice, thereby reducing glucose uptake and lipogenesis [17]. Contrary to these data, cows fed 10 g/day of either cis-9, trans-11 or trans-10, cis-12 CLA for 4 days experienced no change in plasma glucose or insulin levels, although *de novo* lipogenesis was decreased while plasma non-esterified fatty acid levels increased with trans-10, cis-12 supplementation [48]. A similar study in cows supplemented with 100 g/day of mixed CLA isomers for 1 day found a decrease in both *de novo* fatty acid synthesis and fatty acid desaturation [49]. In support of these data, we found that *de novo* lipogenesis [44] and TG esterification [50] decreased with increasing levels of trans-10, cis-12, but not cis-9, trans-11, CLA in cultures of human preadipocytes. Contrary to these data, 3T3-L1 preadipocytes treated with 50 μM trans-10, cis-12 CLA had higher levels of ^{14}C-oleic acid incorporation into both total lipid and specifically triglyceride than either linoleic acid-treated or control cultures [51]. Collectively, these data suggest that trans-10, cis-12 CLA decreases TG content, in part, by decreasing fatty acid synthesis and esterification into TG, at least in certain species and model systems.

Peroxisome proliferation and β-oxidation. 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 rates increase without proportional increases in ATP synthesis, resulting in energy wastage as heat. This energy wastage can lead to reductions in serum and tissue lipids and body fat. Several studies have examined the ability of CLA to increase peroxisomal or mitochondrial activity or fatty acid oxidation. In one such study, rats were fed a diet containing either 0.5–1.5% (w/w) mixed isomers of CLA or a peroxisome proliferator (Wy-14,643) [52]. CLA had little or no effect on body weight, liver weight, or hepatic peroxisome proliferation compared to the Wy-14,643-fed rats. However, both 1.5% CLA and Wy-14,643 increased total liver lipid content. Hepatic fatty acyl-CoA oxidase (ACO; the rate-determining enzyme for peroxisomal β-oxidation) and fatty acid binding protein (L-FABP) mRNA levels were also increased relative to control rats, but not to the degree of Wy-14,643 fed rats. Finally, 100 uM CLA activated a PPAR response element construct through activation of endogenous PPAR subtypes (primarily PPARα and PPARβ-the subtypes found in the liver) expressed in FaO cells (a rat hepatoma cell line). From these results, the authors concluded that mixed isomers of CLA are able to activate PPARα and β, but do not function as peroxisome proliferators per se in this model.

The previous results were confirmed in two more studies by Martha Belury's group [53,54], in which mice fed 0.5–1.5% (w/w) CLA compared to 1.5% linoleic acid had increased levels of ACO, L-FABP, and cytochrome P450IVA1, enzymes that are involved in peroxisome proliferation [53]. *In vitro*, CLA (specifically the cis-9, trans-11 isomer of CLA more so than the trans-10, cis-12 isomer) was also a high affinity ligand for PPARα and PPARβ. Furthermore, the cis-9, trans-11 isomer of CLA induced accumulation of PPAR-responsive mRNAs including ACO, L-FABP, and cytochrome P450IVA1 in a rat hepatoma cell line [54].

The effects of CLA treatment are, to a large extent, reminiscent of the effects observed when animals are treated with PPARα ligands. Although recent data from Pariza's lab using PPARα-null mice indicate that effects of mixed CLA isomers on general body composition were independent of PPARα, disruption of the PPARα gene abolished the CLA-induced activation of a subset of PPARα target genes [53]. Unfortunately, this study did not address isomer-specific effects of CLA, and it is possible that the effects of one isomer was masked by the other. Moreover, the direct effects of CLA on (pre)adipocytes were not investigated. Collectively, these data suggest that CLA in-

creases peroxisomal activity, and that PPARα can indeed mediate some of these effects of CLA at the cellular level.

Several studies support CLA's ability to increase fatty acid oxidation. For example, Sergiel et al. demonstrated that rats oxidized radiolabeled cis-9, trans-11 and trans-10, cis-12 CLA to a much greater extent than radiolabeled linoleic acid over a 24 h period [56]. Similarly, isolated perfused livers from rats fed 1% (w/w) mixed CLA isomers for 2 weeks produced significantly more ketone bodies and less cholesterol compared to isolated livers from 1% (w/w) linoleic acid-fed rats [57]. In addition, the ratio of β-hydroxybutyrate: acetoacetate increased, leading the authors to postulate that dietary CLA appears to exert its hypolipidemic effect by increasing β-oxidation of fatty acids at the expense of fatty acid esterification. In support of this hypothesis, we observed a 55% increase in β-oxidation of ^{14}C-labeled oleic acid in 3T3-L1 preadipocytes treated with 50 μM trans-10, cis-12 CLA for 6 days [51]. In agreement with these data, Martin et al. [58] found that the trans-10, cis-12 isomer of CLA increased hepatic and adipose carnitine palmitoyl-CoA transferase-1 (CPT-1) in rats consuming 1% (w/w) trans-10, cis-12 CLA in the diet for 6 weeks. Moreover, Martin et al. [58] suggest that trans-10, cis-12 CLA, because of its geometric and positional structure, was more efficiently oxidized by enzymes of the β-oxidation pathway than either cis-9, trans-11 CLA or other unsaturated fatty acids. *In vivo,* rats fed mixed CLA isomers had decreased respiratory quotients, indicating they had increased rates of fat oxidation [16,21]. Taken together, these data advance the concept that trans-10, cis-12 CLA decreases TG content, in part, by increasing mitochondrial fatty acid oxidation.

CLA incorporation into cellular lipids. CLA has also been shown to alter the fatty acid profile of various tissues in both animal and cell culture studies. Numerous studies have determined that CLA incorporates into tissues lipids of rodents, chickens, and ruminant animals following dietary CLA consumption [59–63]. However, the cis-9, trans-11 isomer of CLA was found in higher concentrations in tissue lipids than the trans-10, cis-12 isomer. Higher levels of the cis-9, trans-11 CLA isomer were also detected *in vitro* in 3T3-L1 preadipocytes treated with either 50 μM cis-9, trans-11 or trans-10, cis-12 CLA [41]. The significance of this observation is unclear as research has not yet determined if this is the result of increased incorporation of the cis-9, trans-11 isomer into the lipid fractions or increased metabolism of the trans-10, cis-12 isomer. We also found that differentiating cultures of human preadipocytes treated with trans-10, cis-12 CLA for 12 days had 1.0- and 5.5-fold more trans-10, cis-12 CLA in their phospholipid and neutral lipid fractions, respectively, compared to the vehicle (BSA) control cultures [46]. Collectively, these data demonstrate that CLA incorporates into cellular lipids.

CLA's effects on SFA: MUFA ratio. In vivo and *in vitro* studies have determined that CLA treatment alters the ratio of saturated to unsaturated fatty acids, especially the ratio of palmitate:palmitoleate and stearate:oleate. For example, laying hens fed 0.5% (w/w) mixed CLA isomers for 84 days produced eggs with a higher ratio of 16:0/16:1 and 18:0/18:1 compared to control hens [64]. Furthermore, cows consuming 50–100 g/day of mixed CLA isomers for 5 days produced milk with a similar increase in 16:0/16:1 and 18:0/18:1 [61]. Pigs fed 1 to 5% (w/w) mixed CLA isomers had increased levels of SFA and decreased levels of unsaturated fatty acids intramuscularly [65]. Finally, sows fed 2% (w/w) mixed CLA isomers for 35 days had more 16:0 (g/100g lipid) and less 18:1 in their backfat as well as increased 16:0 and decreased 16:1 in their milk compared to controls [60].

Cell culture studies have shown similar CLA-mediated increases in SFA:MUFA ratios. Specifically, Lee et al. [66] found that treatment of H2.35 liver cells with 150 μM mixed CLA isomers for 20 h increased 16:0 and 18:0, while decreasing 18:1 levels compared to control cultures. Comparable results have been observed in 3T3-L1 adipocytes that were treated with 10–100 μM trans-10, cis-12 CLA during differentiation [45]. We found that differentiating cultures of human preadipocytes treated with trans-10, cis-12 CLA for 12 days had significantly higher SFA:MUFA ratios (combined C16 and C18 fatty acids) in their phospholipid and neutral lipid fractions, respectively, compared to control cultures [46]. Taken together, these data demonstrate that CLA incorporates into cellular lipid and alters fatty acid composition of the cultures. Thus, CLA may act to inhibit lipogenesis and TG esterification through a disruption in the fatty acid desaturation process.

One of the key desaturation enzymes involved in lipogenesis is SCD-1 (also known as Δ9 desaturase); an enzyme that catalyzes Δ9-cis desaturation of a number of fatty acid substrates including palmitoyl and stearoyl-CoA. Desaturation of these SFA is necessary to produce MUFA essential for incorporation into the sn-2 position of TG. Since the ratio of SFA:MUFA increases with CLA, inhibition of SCD-1 activity and/or transcription may be one explanation for CLA's lipid-lowering effects [45].

In support of this concept, mice fed 0.5% (w/w) CLA in a diet containing either high carbohydrate or 5.0% (w/w) corn oil experienced a 45 or 75% decrease, respectively, in SCD-1 mRNA levels in the liver compared to controls [66]. The addition of 150 μM of mixed isomers of CLA to H2.35 mouse liver cells also reduced SCD-1mRNA expression. However, this inhibition was not seen when the cells were treated solely with the cis-9, trans-11 isomer of CLA, suggesting isomers other than cis-9, trans-11 CLA (i.e., trans-10, cis-12) inhibit SCD-1 gene expression.

Bretillon et al. [67] determined that 30–120 nM cis-9, trans-11 and trans-10, cis-12 CLA decreased the activity of Δ6-desaturase in rat liver microsomes compared to vehicle treated cultures. However, the trans-10, cis-12 isomer of CLA only reduced the activity of Δ6-desaturase at the highest level (120 nM). Interestingly, Δ9 desaturase activity was inhibited only with trans-10, cis-12 CLA treatment.

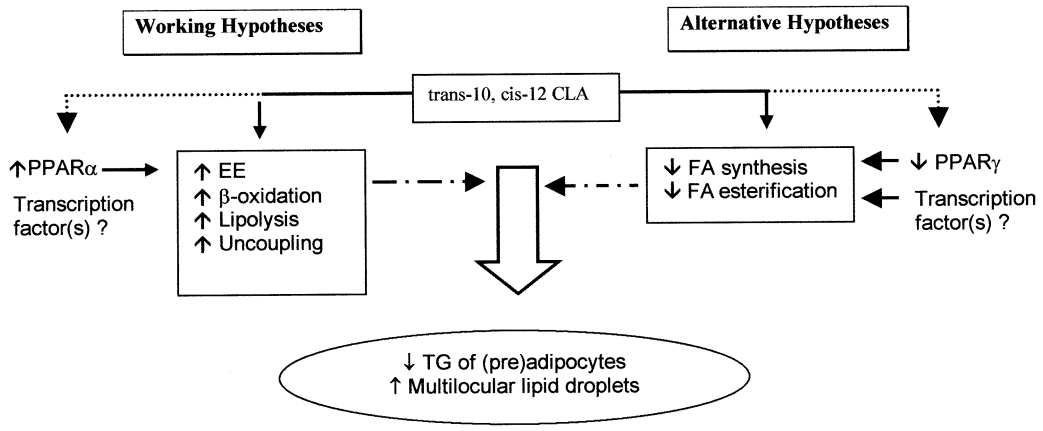

Fig. 2. Our central hypothesis for how conjugated linoleic acid (CLA) decreases adipocyte triglyceride (TG) content. Our working hypothesis is that trans-10, cis-12 attenuates TG content and droplet morphology of primary cultures of human (pre)adipocytes and alters lipid morphology by enhancing energy expenditure (EE), lipolysis, and fatty acid oxidation. We postulate that PPARα activation may be responsible, in part, for up-regulating rate-determining enzymes in peroxisomes and mitochondria. Alternatively, trans-10, cis-12 may decrease PPARγ activity, thereby down-regulating the expression of rate-determining, lipogenic genes controlling lipogenesis and TG esterification.

Another study using rat liver microsomes found that 100 μM mixed isomers of CLA reduced Δ9 desaturase/SCD-1 activity compared to vehicle cultures [68]. Furthermore, the trans-10, cis-12 CLA isomer was responsible for this decrease. These last two studies treated the rat liver microsomes for only 15 min, suggesting that CLA exerts its effect primarily on enzyme activity rather than mRNA or protein synthesis.

Mixed isomers of CLA inhibited either SCD-1 mRNA or activity in liver cells [69] and adipocytes (mixed isomers and specifically trans-10, cis-12 CLA) [45]. Furthermore, Park et al. [68] found that the trans-10, cis-12 isomer of CLA specifically inhibited SCD-1 activity in isolated rat liver microsomes. Thus, CLA appears to exert some of its antilipogenic effects through an isomer specific, desaturation-dependent reduction in the synthesis of MUFAs- essential components for TG and phospholipid synthesis.

CLA, arachidonic acid(AA), and AA-derived eicosanoid synthesis. CLA treatment has been suggested to inhibit the production of adipogenic fatty acids such as linoleic acid, AA, and their eicosanoid metabolites. A reduction in AA and other adipogenic fatty acids may decrease TG esterification, conversion into phospholipids that are critical for cellular metabolism, and/or synthesis into lipid second messengers, such as prostaglandin J_2 (PGJ_2), that may regulate adipogenesis. Several studies have observed such a CLA-mediated decrease in these longer chain fatty acids. For example, Belury and Kempa-Stecko [70] determined that CLA (cis-9, trans-11 and trans-10, cis-12 isomers) incorporated into the hepatic neutral lipids and phospholipids of mice at the expense of linoleic acid and AA. Furthermore, Sugano et al. [71] observed a decrease in hepatic 20:4 concentrations following 1% (w/w) mixed CLA isomer supplementation for 2 weeks in rats. More recently, Stangl [72] found a decrease in both 18:2 and 20:4 in the livers of rats consuming 3% (w/w) mixed CLA isomers for 39 days. In laying hens, the yolks of the hens fed 2.5% mixed CLA isomers also contained lower levels of 18:2, 18:3, and 20:4 compared to control hens [73]. Therefore, CLA may decrease AA-derived cell signals such as prostaglandins and leukotrienes that are critical regulators of cell growth, differentiation, and metabolism.

CLA and lipolysis. Several studies have suggested that CLA alters lipid metabolism by inducing lipolysis. For example, Baumgaurd et al. [48] found that plasma non-esterified fatty acid levels were increased in cows fed 10 g/day trans-10, cis-12 CLA for 4 days, although they did not investigate the mechanism behind this increase. Park et al. [11] observed that mature 3T3-L1 adipocytes treated with 100 μM mixed CLA isomers had higher levels of glycerol release compared to BSA controls. Recent studies have confirmed that the trans-10, cis-12 isomer of CLA was responsible for this increase in glycerol release in murine adipocytes [19,51]. In cultures of human adipocytes, trans-10. cis-12 CLA either increases [74] or has no effect [50] on basal lipolysis. Thus, it appears that CLA may decrease TG content, in part, by increasing basal lipolysis.

5. Potential Antiobesity Mechanism of Action of trans-10, cis-12 CLA (Fig. 2)

CLA decreases adipogenesis. The effects of CLA on adipogenesis and TG content are multifarious, ranging from effects on preadipocyte proliferation to adipocyte differentiation and metabolism (see Figure 2 for mechanisms proposed by the authors). *In vitro* and *in vivo* studies have demonstrated that CLA decreases adipocyte cellularity by decreasing proliferation [39,40] or adipocyte size [21,40,43, 44]. In addition, the trans-10, cis-12 isomer induces apoptosis in mice [20] and 3T3-L1 preadipocytes [41]; an observation that also correlates with a reduction in adipocyte cellularity. CLA has been suggested to decrease adipocyte size and TG content by decreasing differentiation; however, these results are inconsistent. By decreasing

PPARγ expression or activity, CLA may reduce the expression of lipogenic enzymes that control lipogenesis and TG esterification. However, with the exception of decreased LPL expression and activity [11,18,66], the expression of these enzymes and their activities has not been demonstrated.

CLA decreases lipid synthesis. CLA decreases fatty acid desaturation and elongation potentially through a reduction in SCD-1 activity and expression. Decreased SCD-1 activity could lead to an increase in the SFA:MUFA ratio, inhibition of *de novo* fatty acid and TG synthesis, as well as decreased production of pro-adipogenic phospholipids such as PGJ_2 [44–46,66,68].

CLA increases lipolysis. CLA increases basal lipolysis in 3T3-L1 preadipocytes [18,19,51] and human adipocytes [74], leading to a decrease in TG content [48,19,51].

CLA increases energy expenditure and fatty acid oxidation. CLA has diverse effects on lipid metabolism, depending on the isomer type, dose, and duration of treatment and the metabolic status and species of the subject. This includes an induction of β-oxidation in rodents [57,59] and 3T3-L1 preadipocytes [51]. Further support for this concept comes from *in vivo* data from rodent liver [52,53,55,57,58], adipose tissue [58,75], and muscle [20], as well as *in vitro* data from hepatocytes [54], demonstrating that mixed isomers of CLA increased respiratory quotient (RQ), peroxisomal β-oxidation, and the expression of CPT-1, ACO, and UCP-2. Trans-10, cis-12 CLA is believed to be more efficiently oxidized than other unsaturated fatty acids due to its geometric and positional structure. CLA also induced PPARα expression in hepatocytes, which has been postulated to mediate CLA's effects on lipid metabolism [54].

6. Conclusions/implications

Although animal and cell culture experiments seem to clearly support an isomer-specific role for CLA in preventing or reducing adiposity, it is too early to predict the extent to which CLA supplements will be useful in humans. At present, it is known that the effects of CLA on adipogenesis and lipid metabolism in animals are isomer-, dose-, time-, and species-dependent. Specifically, the trans-10, cis-12 isomer seems to be isomer responsible for CLA's antiobesity effects in animals and in (pre)adipocytes from both animals [19,40,41,51] and humans [43,44,46,50]. Furthermore, termination of CLA supplementation results in an increase in TG content or adiposity in 3T3-L1 preadipocytes [41] or rats [18]. Since the effects of CLA seem to be short-lived, it is possible that when an individual stops taking CLA, body fat would likely return to previous, if not higher levels than before CLA consumption. Therefore, long-term supplementation will likely be necessary if CLA were the only treatment used to control adiposity. However, there are no long-term studies on CLA in animals or in humans, making such an endeavor questionable in terms of efficacy and safety.

It is difficult to consume enough CLA in its natural form (i.e., red meats or cheeses) to decrease body fat, especially for vegetarians or individuals consuming a low-fat diet. Moreover, the CLA found in these foods is mostly cis-9, trans-11 CLA, rather than trans-10, cis-12 CLA-the putative antiadipogenic isomer. In addition, dietary fatty acids, especially MUFAs and n-6 fatty acids such as linoleic acid, may reverse CLA's antiobesity effects. Therefore, CLA supplements may be most effective in combination with a fat-reduced diet [30], in physically active subjects [32], or in overweight subjects [30]. Such CLA supplements should be used with caution as they are both expensive and their safety has not been evaluated. In addition, like all nutraceuticals, CLA supplements are not regulated, thus the concentration of CLA may be questionable.

In conclusion, more research is needed, especially among humans, to determine the isomer-specific mechanisms of action and efficacy of CLA as an antiobesity agent. It is especially important to note that the long-term effects of CLA are unknown, thus they should be addressed before recommending CLA supplementation to humans. Furthermore, optimal isomer, timing, and dosage of CLA along with the effects of energy intake and BMI of the subjects on CLA efficacy in humans remain unclear. Lack of such knowledge is of significance as it impedes the development of isomer-specific, CLA fortified foods or supplements for the prevention or treatment of obesity, the most prevalent nutrition-related disease in America.

References

[1] J. Griinari, B. Cori, S. Lacy, P. Chouinard, K. Nurmela, D. Bauman, Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta-9 desaturase, J Nutr 130 (2000) 2285–2291.
[2] H. Lin, T. Boylston, M. Chang, L. Luedecke, T. Shultz, Survey of the conjugated linoleic acid contents of dairy products, J Dairy Sci 78 (1995) 2358–65.
[3] S. Chin, W. Liu, J. Storkson, Y. Ha, M. Pariza, Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens, J Food Comp Anal 5 (1992) 185–197.
[4] M. Britton, C. Fong, D. Wickens, J. Yudkin, Diet as a source of phospholipid esterified 9,11-octadecandienoic acid in humans, Clin Sci 83 (1992) 97–101.
[5] A. Fogerty, G. Ford, D. Svoronos, Octadeca-9–11-dienoic acid in foodstuff and in the lipids of human blood and breast milk, Nutr Reports Int 38 (1988) 937–944.
[6] Y. Ha, N. Grimm, M. Pariza, Anticarcinogens from fried ground beef: heat altered derivatives of linoleic acid, Carcinogenesis 8 (1987) 1881–1887.
[7] Y. Ha, J. Storkson, M Pariza, Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated derivatives of linoleic acid, Cancer Res 50 (1990) 1097–1101.
[8] C. Miller, Y. Park, M. Pariza, M. Cook, Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection, Biochem Biophys Res Commun 198 (1994) 1107–1112.
[9] K. Lee, D. Kritchevsky, M. Pariza, Conjugated linoleic acid and atherosclerosis in rabbits, Atherosclerosis 108 (1994) 19–25.
[10] S. Chin, J. Storkson, K. Albright, M. Cook, M. Pariza, Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency, J Nutr 124 (1994) 2344–2349.

[11] Y. Park, K. Albright, W. Liu, J. Storkson, M. Cook, M. Pariza, Effect of conjugated linoleic acid on body composition in mice, Lipids 32 (1997) 853–858.
[12] R. Devery, A. Miller, C. Stanton, Conjugated linoleic acid and oxidative behaviour in cancer cells, Biochem Soc Trans 29 (2001) 341–344.
[13] M. Pariza, Y. Park, M. Cook, The biologically active isomers of conjugated linoleic acid, Prog Lipid Res 40 (2001) 283–298.
[14] R. Nicolosi, E. Rogers E, D. Kritchevsky, J. Scimeca, P. Huth, Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters, Artery 22 (1997) 266–277.
[15] K. Houseknecht, J. Heuvel, S. Moya-Camarena, C. Portocarrero, L. Peck, K. Nickel, M. Belury, Dietary conjugated linoleic acid normalizes impaired glucose tolerance in zucker diabetic fatty fa/fa rat, Biochem Biophys Res Commun 244 (1998) 678–682.
[16] D. West, J. Delany, P. Camet, F. Blohm, A. Truett, J. Scimeca, Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse, Am J Physiol 275 (1998) R667–R672.
[17] J. DeLany, F. Blohm, A. Truett, J. Scimeca, D. West, Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake, Am J Physiol 276 (1999) R1172–R1179.
[18] Y. Park, J. Storkson, K. Albright, W. Liu, M. Pariza, Evidence that trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice, Lipids 34 (1999) 235–41.
[19] Y. Park, K. Albright, J. Storkson, W. Liu, M. Cook, M. Pariza, Changes in body composition in mice during feeding and withdrawl of conjugated linoleic acid, Lipids 34 (1999) 243–248.
[20] N. Tsuboyama-Kasaoka, M. Takahashi, K. Tanemura, H. Kim, T. Tange, H. Okuyama, M. Kasai, S. Ikemoto, O. Ezaki, Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice, Diabetes 49 (2000) 1534–1542.
[21] M. Azain, D. Hausman, M. Sisk, W. Flatt, D. Jewell, Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number, J Nutr 130 (2000) 1548–1554.
[22] J. DeLany, D. West, Changes in body composition with conjugated linoleic acid, J Am Coll Nutr 19 (2000) 478S–493S.
[23] D. West, F. Blohm, A. Truett, J. DeLany, Conjugated linoleic acid persistently increases total energy expenditure in akr/j mice without increasing uncoupling protein gene expression, J Nutr 130 (2000) 2471–2477.
[24] M. Cook, D. Jerome, T. Crenshaw, P. Buege, M. Pariza., K. Albright, S. Schmidt, J. Scimeca, P. Lotgren, E. Hentges, Feeding conjugated linoleic acid improves feed efficiency and reduces carcass fat in pigs, Adipocyte Biology and Hormone Signaling Symposium. University of Wisconsin-Madison, Dept. Biochemistry (1999) P67.
[25] E. Ostrowska, M. Muralitharan, R. Cross, D. Bauman, F. Dunshea, Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs, J Nutr 129 (1999) 2037–2042.
[26] C. Niesler, B. Urso, J. Prins, K. Siddle, IGF-I inhibits apoptosis induced by serum withdrawal, but potentiates tnf-alpha-induced apoptosis, in 3T3–L1 preadipocytes, J Endocrinol 167 (2000) 165–74.
[27] M. Yamasaki, K. Mansho, H. Mishima, M. Kasai, M. Sugano, H. Tachibana, K. Yamada, Dietary effect of conjugated linoleic acid on lipid levels in white adipose tissue of Sprague-Dawley rats, Biosci Biotechnol Biochem 63 (1999) 1104–1106.
[28] H. Muller, G. Stangl, M. Kirchgessner, Energy balance of conjugated linoleic acid-treated pigs, J Anim Physiol and Anim Nutr 81 (1999) 150–156.
[29] A. Buison, F. Ordiz, M. Pillizzon, K. Jen, Conjugated linoleic acid does not impair fat regain but alters igf-1 levels in weight-reduced rats, Nutr Res 20 (2000) 1591–1601.
[30] H. Blankson, J. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, O. Gudmundsen, Conjugated linoleic acid reduces body fat mass in overweight and obese humans, J Nutr 130 (2000) 2943–2948.
[31] A. Smedman, G. Vessby, Conjugated linoleic acid supplementation in humans—metabolic effects, Lipids 36 (2001) 773–781.
[32] E. Thom, J. Wadstein, O. Gudmundsen, Conjugated linoleic acid reduces body fat in healthy, exercising humans, J Int Med Res 29 (2001) 392–396.
[33] K. Zambell, N. Keim, M. Van Loan, B. Gale, P. Benito, D. Kelley, G. Nelson, Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure, Lipids 35 (2000) 777–782.
[34] K. Zambell, W. Horn, N. Keim, Conjugated linoleic acid supplementation in humans: effects on fatty acids and glycerol kinetics, Lipids 36 (2001) 767–772.
[35] E. Medina, W. Horn, N. Keim, P. Havel, P. Benito, D. Kelley, G. Nelson, K. Erickson, Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite, Lipids 35 (2000) 783–788.
[36] G. Berven, A. Bye, O. Hals, H. Blankson, H. Fagertun, E. Thom, J. Wadstein, O. Gudmundsen, Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers, European J Lipid Sci Technol 102 (2000) 455–462.
[37] V. Gavino, G. Gavino, M. LeBlanc, B. Tuchweber, An isomeric mixture of conjugated linoleic acids but not pure cis-9, trans-11-octadecodienoic acid affects body weight gain and plasma lipids in hamsters, J Nutr 130 (2000) 27–29.
[38] D. Satory, S. Smith, Conjugated linoleic acid inhibits proliferation but stimulates lipid filling of murine 3t3–l1 preadipocytes, J Nutr 129 (1999) 92–97.
[39] A. Brodie, V. Manning, K. Ferguson, D. Jewell, C. Hu, Conjugated linoleic acid inhibits differentiation of pre- and post-confluent 3t3–l1 preadipocytes but inhibits cell proliferation only in pre-confluent cells, J Nutr 129 (1999) 602–606.
[40] M. Evans, C. Geigerman, J. Cook, L. Curtis, B. Kuebler, M. McIntosh, Conjugated linoleic acid suppresses triglyceride content and induces apoptosis in 3t3–l1 preadipocytes, Lipids 35 (2000) 899–910.
[41] M. Evans, C. Geigerman, L. Curtis, B. Keubler, Y. Park, M. Pariza, M. McIntosh, Trans-10, cis-12 conjugated linoleic acid reduces triglyceride content while differentially affecting pparγ2 and ap2 expression in 3t3–l1 preadipocytes, Lipids 36 (2001) 1223–1232.
[42] M. Igarashi, T. Miyazawa, The Growth Inhibitory Effect of conjugated linoleic acid on a human hepatoma cell line, hepg2, is induced by a change in fatty acid metabolism, but not the facilitation of lipid peroxidation in the ce1ls, Biochim Biophys Acta 1530 (2001) 162–71.
[43] J. Brown, M. Evans, M. McIntosh, Linoleic acid partially reverses the suppressive effects of conjugated linoleic acid on preadipocyte triglyceride content, J Nutr Biochem 12 (2001) 381–387.
[44] J. Brown, Y-D. Halverson, R. Lea-Currie, C. Geigerman, M. McIntosh, Trans-10, cis-12, but not cis-9, trans-11, conjugated linoleic acid attentuates lipogenesis in primary cultures of stromal vascular cells from human adipose tissue, J Nutr 131 (2001) 2316–2321.
[45] Y. Choi, Y. Kim, Y. Han, Y. Park, M. Pariza, J. Ntambi, The trans-10, cis-12 isomer of conjugated linoleic acid downregulates stearoyl-coa desaturase 1 gene expression in 3t3–l1 adipocytes, J Nutr 130 (2000) 1920–1924.
[46] J. Brown, O. Fabiyi, R. Lea-Currie, J. Storkson, M. Pariza, M. McIntosh, Trans-10, Cis-12 Conjugated linoleic acid decreases triglyceride content by altering lipid metabolism, but not pparγ or c/ebpα expression, in primary cultures of human (pre)adipocytes, FASEB J 16 (2002) 1015.
[47] S. Ding, R. McNeel, H. Mersmann, Conjugated linoleic acid increases the differentiation of porcine adipocytes *in vitro*, Nutr Res 20 (2000) 1569–1580.
[48] L. Baumgard, B. Corl, D. Dwyer, A. Saebo, D. Bauman, Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis, Am J Physiol 278 (2000) R179–R184.
[49] J. Loor, J. Herbein, Exogenous conjugated linoleic acid isomers reduce milk fat concentration and yield by inhibiting *de novo* fatty acid synthesis, J Nutr 128 (1998) 2411–2419.
[50] J. Brown, Y. Lea-Currie, A. Sen, M. McIntosh, Trans-10, cis-12 conjugated linoleic acid suppress fatty acid incorporation into lipid in

cultures of human preadipocytes, Obesity Res 9 (2001) 142S (Abstract #PE8).
[51] M. Evans, X. Lin, J. Odle, M. McIntosh, Trans-10, cis-12 conjugated linoleic acid increases fatty acid oxidation in 3t3–l1 preadipocytes, J Nutr 132 (2002) 450–455.
[52] S. Moya-Camarena, J. Vanden Heuvel, M. Belury, Conjugated linoleic acid activates peroxisome proliferators-activated receptor α and β subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats, Biochimica et Biophysica Acta 1436 (1999) 331–342.
[53] M. Belury, S. Moya-Camarena, K. Liu, J. Vanden Huevel, Dietary conjugated linoleic acid induces peroxisome-specific enzyme accumulation and ornithine decarboxylase activity in mouse liver, J Nutr Biochem 8 (1997) 579–584.
[54] S. Moya-Camarena, J. Vanden Heuvel, S. Blanchard, L. Leesnitzer, M. Belury, Conjugated linoleic acid is a potent naturally occurring ligand and activator of pparα, J Lipid Res 40 (1999) 1426–1433.
[55] J. Peters, Y. Park, F. Gonzalez, M. Pariza, Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator activated receptor-alpha null mice, BBA 1533 (2001) 233–242.
[56] J. Sergiel, J. Chardigny, J. Sebedio, O. Berdeaux, P. Juaneda, O. Loreau, B. Pasquis, J. Noel, Beta-oxidation of conjugated linoleic acid isomers and linoleic acid in rats, Lipids 36 (2001) 1327–1329.
[57] M. Sakano, F. Miyanaga, S. Kawahara, K.Yamauchi, N. Fukuda, K. Wantabe, T. Iwata, M. Sugano, Dietary conjugated linoleic acid reciprocally modifies ketogenesis and lipid secretion by the rat liver, Lipids 34 (1999) 997–1000.
[58] J. Martin, S. Gregoire, M. Seiss, M. Genty, J. Chardigny, O. Berdeaux, P. Juaneda, J. Sebedio, Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats, Lipids 35 (2000) 91–98.
[59] E. de Deckere, J. van Amelsvoort, G. McNeill, P. Jones, Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster, Brit J Nutr 82 (1999) 309–317.
[60] G. Bee G Dietary conjugated linoleic acids alter adipose tissue and milk lipids of pregnant and lactating sows, J Nutr 130 (2000) 2292–2298.
[61] P. Chouinard, L. Courneau, D. Barbano, L. Metzger, D. Bauman, Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows, J Nutr 129 (1999) 1579–1584.
[62] O. Simon, K. Manner, K. Schafer, A. Sagredos, K. Eder, Effects of conjugated linoleic acids on protein to fat proportions, fatty acids, and plasma lipids in broilers, Eur J Lipid Sci Technol 102 (2000) 402–410.
[63] B. Szymczyk, P. Pisulewski, W. Szczurek, P. Hanczakowski, The effects of feeding conjugated linoleic acid (CLA) on rat growth performance, serum lipoproteins and subsequent lipid composition of selected rat tissues, J Sci Food Agr 80 (2000) 1553–1558.
[64] R. Aydin, M. Pariza, M. Cook, Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality, J Nutr 131 (2000) 800–806.
[65] S. Joo, J. Lee, Y. Ha, G. Park, Effects of dietary conjugated linoleic acid on fatty acid composition, lipid oxidation, color, and water-holding capacity of pork loin, J Anim Sci 80 (2002) 108–112.
[66] K. Lee, M. Pariza, J. Ntambi, Conjugated linoleic acid decreases hepatic stearoyl-coa desaturase mrna expression, Biochem Biophys Res Commun 248 (1998) 817–821.
[67] L. Bretillon, J. Chardigny, S. Gregoire, O. Berdeaux, J. Sebedio, Effects of conjugated linoleic acid isomers on the hepatic microsomal desaturation activities *in vitro*, Lipids 34 (1999) 965–969.
[68] Y. Park, J. Storkson, J. Ntambi, M. Cook, C. Sih, M. Pariza, Inhibition of hepatic stearoyl-coa desaturase activity by trans-10, cis-12 conjugated linoleic acid and its derivatives, Biochim Biophys Acta 1486 (2000) 285–292.
[69] D. Cunningham, L. Harrison, T. Shultz, Proliferative responses of normal human mammary and mcf-7 breast cancer cells to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture, Anticancer Res 17 (1997) 197–204.
[70] M. Belury, A. Kempa-Stecko, Conjugated linoleic acid modulates hepatic lipid composition in mice, Lipids 32 (1997) 199–204.
[71] M. Sugano, A. Tsujita, M. Yamasaki, M. Noguchi, K. Yamada, Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats, Lipids 33 (1997) 521–527.
[72] G. Stangl, Conjugated linoleic acids exhibit a strong fat-to-lean partitioning effect, reduce serum vldl lipids and redistribute tissue lipids in food-restricted rats, J Nutr 130 (2000) 1140–1146.
[73] M. Du, D. Ahn, J. Sell, Effects of dietary conjugated linoleic acid and linoleic: linolenic acid ratio on polyunsaturated fatty acid status in laying hens, Poultry Sci 79 (2000) 1749–1756.
[74] D. Sujkowska, C. Auchter, D. Franklin, N. Ellis, Y. Lea-Currie, A. Sen, Effects of conjugated linoleic acid isoforms on lipolysis and leptin secretion from cultured human adipocytes, Obesity Res 9 (2001) 147 (Abstract #P#28)
[75] S. Rahman, Y. Wang, H. Yotsumoto, J. Cha, S. Han, S. Inoue, T. Yanagita, Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and beta-oxidation of fatty acid in oletf rats, Nutrition 17 (2001) 385–90.
[76] M. Yamasaki, K. Mansho, Y. Ogino, M. Kasai, H. Tachibana, K. Yamada, Acute reduction of serum leptin level by dietary conjugated linoleic acid in Sprague-Dawley rats, J Nutr Biochem 11 (2000) 467–471.
[77] J. Kramer, N. Sehat, M. Dugan, M. Mossoba, M. Yurawecz, J. Roach, K. Eulitz, J. Aalhus, A. Schaefer, Y. Ku, Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography, Lipids 33 (1998) 549–58.
[78] H. Muller, M. Kirchgessner, F. Roth, G. Stangl, Effect of conjugated linoleic acid on energy metabolism in growing-finishing pigs, J Anim Physiol Anim Nutr 83 (2000) 85–94.
[79] J. Bassaganya-Riera, R. Hontecillas-Magarzo, K. Bregendahl, M. Wannemuehler, D. Zimmerman, Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence, J Anim Sci 79 (2001) 714–721.
[80] C. Yeung, L. Yang, Y. Huang, J. Wang, Z. Chen, Dietary conjugated linoleic acid mixture affects the activity of intestinal acyl coenzyme a: cholesterol acyltransferase in hamsters, Brit J Nutr 84 (2000) 935–941.
[81] M. Latour, A. Devitt, R. Meunier, J. Stewart, B. Watkins, Effects of conjugated linoleic acid. 1. Fatty acid modification of yolks and neonatal fatty acid metabolism, Poultry Sci 79 (2000) 817–821.
[82] M. Latour, A. Devitt, R. Meunier, J. Stewart, B. Watkins, Effects of conjugated linoleic acid. 2. embryonic and neonatal growth and circulating lipids, Poultry Sci 79 (2000) 822–826.
[83] S. Jones, D. Ma, R. Robinson, C. Field, M. Clandinin, Isomers of conjugated linoleic acid (CLA) are incorporated into egg yolk lipids by CLA-fed laying hens, J Nutr 130 (2000) 2002–2005.
[84] Z. Mir, M. Rushfeldt, P. Mir, L. Paterson, R. Weselake, Effect of dietary supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA content of lamb tissues, Sm Rum Res 36 (2000) 25–31.
[85] R. Twibell, B. Watkins, L. Rogers, P. Brown, Effects of dietary conjugated linoleic acids on hepatic and muscle lipids in hybrid striped bass, Lipids 35 (2000) 155–61.
[86] H. Cantwell, R. Devery, M. O'Shea, C. Stanton, The effect of conjugated linoleic acid on the antioxidant enzyme defense system in rat hepatocytes, Lipids 34 (1999) 833–839.
[87] W. Jun, H. Yang, B. Cho, Comparison of conjugated linoleic acid and linoleic acid on cytotoxicity, synthesis, and secretion of lipids in hepg2 cells, *In Vitro* and Molecular Toxicology 13 (2000) 181–189.
[88] K. Liu, M. Belury, Conjugated linoleic acid modulation of phorbol ester-induced events in murine keratinocytes, Lipids 32 (1997) 725–730.
[89] K. Liu, M. Belury, Conjugated linoleic acid reduces arachidonic acid content and pge_2 synthesis in murine keratinocytes, Cancer Lett 127 (1998) 15–22.