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REVIEWS: CURRENT TOPICS

Antiobesity mechanisms of action of conjugated linoleic acid

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Abstract

Conjugated linoleic acid (CLA), a family of fatty acids found in beef, dairy foods and dietary supplements, reduces adiposity in several animal models of obesity and some human studies. However, the isomer-specific antiobesity mechanisms of action of CLA are unclear, and its use in humans is controversial. This review will summarize *in vivo* and *in vitro* findings from the literature regarding potential mechanisms by which CLA reduces adiposity, including its impact on (a) energy metabolism, (b) adipogenesis, (c) inflammation, (d) lipid metabolism and (e) apoptosis.

Keywords: CLA; Obesity; Adipose tissue; Energy metabolism; PPARγ; Adipogenesis; Inflammation

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of conjugated octadecadienoic acid isomers derived from linoleic acid, a fatty acid that contains 18 carbons and 2 double bonds in *cis* configuration at the 9th and 12th carbons (i.e., *cis*-9,*cis*-12 octadecadienoic acid). Microbes in the gastrointestinal tract of ruminant animals convert linoleic acid into different isoforms of CLA through biohydrogenation. This process changes the position and configuration of the double bonds, resulting in a single bond between one or both of the two double bonds [i.e., *cis*-9,*trans*-11 (9,11) or *trans*-10,*cis*-12 (10,12) octadecadienoic acid].

Commercial preparations of CLA are made from the linoleic acid of safflower or sunflower oils under alkaline conditions. This type of processing yields a CLA mixture containing approximately 40% of the 9,11 isomer and 44% of the 10,12 isomer (reviewed in Pariza et al. [1]). Commercial preparations also contain approximately 4–10% *trans*-9, *trans*-11 CLA and *trans*-10,*trans*-12 CLA, as well as trace amounts of other isomers.

The 9,11 isomer, also known as rumenic acid, is the predominant form of CLA found in naturally occurring foods. 9,11-CLA comprises approximately 90% of CLA found in ruminant meats and dairy products, and the 10,12 isomer comprises the remaining 10%. Although several other isoforms of CLA have been identified (i.e., *trans*-9,*trans*-11, *cis*-9,*cis*-11, *trans*-10,*trans*-11 and *cis*-10,*cis*-12), the 9,11 and 10,12 isomers appear to be the most biologically active [2].

The proportion of CLA ranges from 0.34% to 1.07% of the total fat in dairy products, and from 0.12% to 0.68% of the total fat in raw or processed beef products (reviewed in Dhiman et al. [3], Silveira et al. [4] and Mendis et al. [5]). However, the CLA content of food is dependent on several factors, including the season and the animal's breed, nutritional status and age (reviewed in Dhiman et al. [3]). The average daily intake of CLA is approximately 152–212 mg for nonvegetarian women and men, respectively [6], and human serum levels range from 10 to 70 μmol/L [7,8].

1.1. Antiobesity properties of CLA

CLA was initially discovered in 1987 by Ha et al. [9], and it was first identified as an anticarcinogen. Subsequently, CLA was shown to exhibit antiatherosclerotic (reviewed in Mitchell and McLeod [10]) and antiobesity (reviewed in Whigham et al. [11]) properties. Due to the substantial rise in the prevalence of obesity over the past 30 years [12], interest in CLA as a weight loss treatment has increased. Supplementation with a CLA mixture (i.e., equal concentrations of the 10,12 and 9,11 isomers) or the 10,12 isomer alone decreases body fat mass (BFM) in many animal studies and some human studies (reviewed in Whigham et al. [11] and Wang and Jones [13]). Of the two major isomers of CLA, the 10,12 isomer is specifically responsible for the antiobesity effects [14–18].

1.2. CLA regulation of body weight

Park et al. [19] were the first to demonstrate that CLA modulated body composition. In their study, male and female mice given a 0.5% (wt/wt) CLA mixture had 57% and 60% lower BFM, respectively, than controls. Other researchers have subsequently demonstrated that

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CLA supplementation consistently reduces BFM in mice, rats and pigs [20–24]. For example, dietary supplementation with 1% (wt/wt) CLA mixture for 28 days decreased body weight and periuteral white adipose tissue (WAT) mass in C57BL/6J mice [25]. Similarly, a 1.0–1.5% (wt/wt) mixture of CLA for 3–4 weeks decreased body weight and WAT mass in male ob/ob [26] and ICR [27] obese mice.

Studies investigating CLA's effects on BFM reduction in humans have produced less consistent results. Whereas some studies show that CLA decreases BFM and increases lean body mass (LBM) (reviewed in Wang and Jones [13] and House et al. [16]), others have shown no effect of CLA supplementation on body composition in humans (reviewed in Wang and Jones [13]). For example, supplementation of a CLA mixture in overweight and obese people (3–4 g/day for 24 weeks) decreased BFM and increased LBM [28]. On the other hand, supplementation of CLA mixture in yogurt in healthy adults (3.76 g/day for 14 weeks) had no effect on body composition [29]. In addition, Larsen et al. [30] investigated the potential role of CLA in preventing body weight regain in moderately obese subjects who had lost approximately 10 kg after an 8-week dietary intervention on a low-calorie diet. Supplementation for 1 year with a CLA mixture did not prevent body weight regain compared to controls. Finally, supplementation with 3.2 g/day of a CLA mixture decreased total BFM and trunk fat compared to placebo in overweight subjects, but not in obese subjects [31]. These contradictory findings among human studies may be due to the following differences in experimental design: (a) CLA isomer combination versus individual isomers, (b) CLA dose and duration of treatment, and (c) the gender, weight, age and metabolic status of the subjects.

The primary discrepancy between animal and human studies appears to be the dose of CLA administered. For example, moderately overweight humans with an average weight of 72.5 kg supplemented with a CLA mixture (3.76 g/day for 14 weeks) experienced no decrease in body weight, BMI or BFM [29]. In contrast, C57BL/6 mice supplemented with 1.5% (wt/wt) CLA mixture for 4 weeks weighed significantly less and had reduced adiposity compared to controls [32]. However, while subjects in the human study received approximately 0.05 g/kg body weight CLA, the mice received 1.07 g/kg body weight CLA, which was 20 times the human dose based on body weight. Supplementing humans with higher doses of CLA would address this dosing issue.

Because CLA has the potential to reduce BFM when given at high enough doses and is being taken as a supplement for that purpose, it is important to understand its mechanism of action. Therefore, this review will examine potential mechanisms by which the CLA mixture or 10,12-CLA alone reduces adiposity, with particular emphasis on its effects on WAT. Potential mechanisms to be discussed include regulation of (a) energy metabolism, (b) adipogenesis, (c) inflammation, (d) lipid metabolism and (e) apoptosis.

2. Antiobesity mechanisms of CLA

2.1. CLA regulation of energy metabolism

2.1.1. CLA decreases energy intake

Energy balance is a function of energy intake relative to energy expenditure. When energy intake exceeds energy expenditure, body weight and BFM increase and vice versa. Accordingly, potential mechanisms by which CLA reduces BFM include decreasing energy intake or increasing energy expenditure. Park et al. [19] demonstrated that mice supplemented with a CLA mixture or enriched 10,12-CLA for 4 weeks reduced their food intake. A number of subsequent studies in rodents produced similar results [16,33–36]. No study to date has demonstrated that CLA decreases food intake in humans [37–40].

A mechanism for this reduced food intake was suggested by So et al. [36], who reported that food intake was reduced by 23.6% in mice fed a low-fat diet supplemented with 10,12-CLA. In this study, mice receiving 10,12-CLA had a decreased gene expression ratio of proopiomelanocortin to neuropeptide Y in the hypothalamus. These results suggest that CLA exerts an effect on hypothalamic appetite-regulating genes. In support of this hypothesis, injection of mixed isomers of CLA to the rat hypothalamus reduced the expression of neuropeptide Y and agouti-related protein — neuropeptides that robustly increase food intake [41]. Alternatively, CLA supplementation may reduce food intake by affecting the palatability of the diet; however, so far, there have been no reports supporting this hypothesis.

A number of studies have reported reduced adiposity without changes in energy intake following administration of a CLA mixture in mice [42–45]. For example, supplementation of mice with a CLA mixture for 42 days decreased total body weight without reducing food intake [45]. These data indicate that CLA effects on body fat are not solely dependent on reduction in food or reduction in energy intake. Thus, although several studies show that CLA decreases energy intake, others show no effect, suggesting that CLA can decrease body fat independent of reduction of energy intake.

2.1.2. CLA increases energy expenditure

Energy expenditure is a function of basal metabolic rate (BMR), adaptive thermogenesis, and physical activity. CLA has been proposed to reduce adiposity by elevating energy expenditure via increased BMR, thermogenesis or lipid oxidation in animals [33,43–47]. For example, in BALB/c male mice fed mixed isomers of CLA for 6 weeks, body fat was decreased by 50% compared to controls and was accompanied by increased BMR [45]. Enhanced thermogenesis may be associated with an up-regulation of uncoupling proteins (UCPs), which facilitate proton transport over the inner mitochondrial membrane, thereby diverting energy from ATP synthesis to heat production. UCP1 was the first member of the family to be isolated and is exclusively expressed in brown adipose tissue. UCP3 is expressed in muscle and in a number of other tissues. UCP2 is expressed in a variety of tissues, including WAT, and is the most highly expressed UCP. Supplementation with a CLA mixture or 10,12-CLA in rodents has been shown to induce UCP2 transcription in WAT [27,35,48–50], but whether this plays a role in energy dissipation is unclear. CLA also increased the expression of another mitochondrial protein, carnitine palmitoyltransferase 1 (CPT1), in WAT of 10,12-CLA-treated mice [50,51]. CPT1 is involved in mitochondrial fatty acid uptake and catalyzes the rate-limiting step of fatty acid oxidation. Consistent with these findings, 10,12-CLA increased β-oxidation in differentiating 3T3-L1 mouse preadipocytes [52]. Similarly, CLA supplementation has been shown to increase UCP expression and β-oxidation in rodent muscle and liver [35,53–57].

On the other hand, results from human studies concerning CLA regulation of energy expenditure are mixed. For example, a recent investigation of human supplementation with a CLA mixture (3.9 g/day for 12 weeks) revealed no change in BMR or BFM [39]. Similar results have been reported in other studies of humans supplemented with a CLA mixture [37,58]. In contrast, healthy moderately overweight humans consuming a CLA mixture in yogurt (3.76 g/day for 14 weeks) exhibited higher BMR, although body weight was not affected [29]. Similarly, supplementation with a CLA mixture for 13 weeks increased the resting metabolic rate and fat-free mass in human subjects without a corresponding effect on BFM [59]. Thus far, only one human study has demonstrated both increased energy expenditure and decreased body weight in humans. In this study by Close et al. [60], subjects supplemented with a CLA mixture (4 g/day for 6 months) had decreased body weight and exhibited increased fat oxidation and energy expenditure while sleeping.

Other studies have demonstrated that CLA supplementation increases LBM, which is associated with higher levels of energy expenditure. For example, mixed CLA isomers (6.4 g/day for 12 weeks) increased LBM by 0.64 kg in healthy obese humans compared to controls [58]. Similarly, mice fed a 0.4% (wt/wt) CLA mixture exhibited increased LBM compared to controls [61]. Proposed mechanisms by which CLA increases LBM occur via increased bone or muscle mass, which is supported by evidence from rodent studies. A 10-week 10,12-CLA supplementation [0.5% (wt/wt) mixed isomers] increased bone mineral density and muscle mass in C57BL/6 female mice [62]. CLA supplementation is thought to increase bone mineral density by up-regulating osteogenic gene expression and by down-regulating osteoclast bone-resorbing activity [62,63]. Similarly, CLA supplementation alone or with exercise increased bone mineral density in middle-aged female mice compared to controls [64].

Alternatively, CLA may suppress the adipogenesis of pluripotent mesenchymal stem cells (MSCs) in bone marrow and instead enhance their commitment to become osteoblasts (bone-forming cells). Indeed, 10,12-CLA has been shown to preferentially promote the differentiation of human MSCs into osteoblasts in culture [65]. In contrast, 9,11-CLA increased adipocyte differentiation and decreased osteoblast differentiation. Consistent with these *in vitro* data, CLA mixture supplementation of rats treated with corticosteroids, which decrease muscle and bone mass, prevented reductions in LBM, bone mineral density, and bone mineral content [66]. Collectively, these findings suggest that CLA may reduce adiposity through increased energy expenditure via increased mitochondrial uncoupling and fatty acid oxidation in WAT, or via increased muscle or bone mass. However, the extent to which CLA regulates BMR or LBM and how this contributes to the reduction in body weight or fat in humans remain to be determined.

2.2. CLA regulation of adipogenesis

2.2.1. CLA inhibits adipogenesis

The conversion of preadipocytes into adipocytes involves the activation of key transcription factors such as peroxisome proliferator-activated receptor γ (PPARγ) and CAAT/enhancer binding protein (C/EBP). During the differentiation process, increased C/EBPβ and C/EBPδ activity induces the transcription of C/EBPα and PPARγ, the master regulators of adipocyte differentiation (Fig. 1). There is much evidence showing that CLA suppresses preadipocyte differentiation in animal [18,52,67–71] and human [72,73] preadipocytes. 10,12-CLA treatment has been reported to reduce adipogenesis and lipogenesis specifically by attenuating PPARγ, C/EBPα, sterol regulatory element binding protein (SREBP) 1c, liver X receptor (LXR) α, and adipocyte-specific fatty acid binding protein (aP2) expression [27,71–75].

In rodents, 10,12-CLA supplementation decreased the expression of PPARγ and its target genes [24,50,71,76]. In mature *in vitro* differentiated primary human adipocytes or in mature 3T3-L1 adipocytes, 10,12-CLA treatment leads to a substantial decrease in the expression and activity of PPARγ [18,77] and a decrease in PPARγ target genes and lipid content [73]. These and numerous other studies show that 10,12-CLA specifically is not only able to inhibit but also able to reverse the adipogenic process, and that this may in part be mediated by suppression of PPARγ activity (Fig. 2). The decrease in PPARγ target gene expression may be due to reduced PPARγ expression or posttranslational inhibition of PPARγ activity *per se*. Because PPARγ directly or indirectly induces its own expression, decreased PPARγ activity would be expected to suppress PPARγ expression, making it difficult to determine the level at which inhibition occurs. Time-course experiments conducted in our laboratories, however, indicate that inhibition of PPARγ activity occurs prior to a decrease in PPARγ expression [73], suggesting that inhibition of PPARγ activity may be a primary event.

There are many mechanisms by which CLA may posttranslationally regulate PPARγ. Transient transfection assays with ectopically expressed PPARγ have been employed to assess CLA isomers as potential ligands for PPARγ. In such assays, both CLA isomers have been shown to only modestly activate PPARγ, even at high concentrations. However, they are able to effectively inhibit the action of full agonists such as rosiglitazone and darglitazone [18,70,77,78], indicating that CLA may act as a low-affinity partial agonist. Nonetheless, this mechanism cannot fully account for the 10,12-CLA-specific repression of PPARγ activity.

PPARγ activity may also be regulated by phosphorylation (Fig. 2), which can be mediated by the mitogen-activated protein kinase (MAPK) pathway [79–81]. Ser112 phosphorylation of PPARγ2, the form of PPARγ required for adipocyte differentiation, may decrease its activity via ubiquitination and proteasome degradation [82], and via reduction in both its ligand-dependent and its ligand-independent transactivating functions [80,83–85]. We have demonstrated that 24-h treatment with 10,12-CLA increases PPARγ phosphorylation [77] without significantly decreasing its protein levels, suggesting that the down-regulation of PPARγ target genes is due to decreased transactivating function. Intriguingly, robust extracellular-signal-regulated kinase (ERK) phosphorylation is also observed 24 h after CLA stimulation, suggesting a role for ERK in PPARγ phosphorylation and inactivation. Consistent with these data, we demonstrated that ERK activation is a key player in CLA's suppression of adipogenic gene expression and insulin-stimulated glucose uptake [73]. Therefore, it is tempting to speculate that CLA antagonizes PPARγ activity via activation of MAPKs such as ERK, thereby leading to repression of PPARγ target genes (Fig. 2).

Finally, CLA may interfere with PPARγ activity by virtue of its proinflammatory effects on adipocytes (Fig. 3). We have shown that 10,12-CLA induces nuclear factor κB (NFκB) activation in adipocytes, and that this induction leads to an increased expression of proinflammatory cytokines [86,87]. In addition, NFκB or other proinflammatory transcription factors may interfere directly with PPARγ activation of target genes (Figs. 1–3). This will be discussed in more detail below.

2.3. CLA increases inflammation

Although the primary function of WAT is energy storage, it also has the ability to produce a number of proinflammatory cytokines. These adipokines (i.e., cytokines produced by adipose tissue) can cause insulin resistance (Fig. 4), thereby suppressing lipid synthesis and increasing lipolysis in adipocytes (Fig. 5). Induction of these inflammatory genes is dependent on various cellular kinases, including MAPK, and is driven by transcription factors such as NFκB, which have been reported to directly antagonize PPARγ (Figs. 1–3). Tumor necrosis factor α (TNFα), in particular, exerts potent

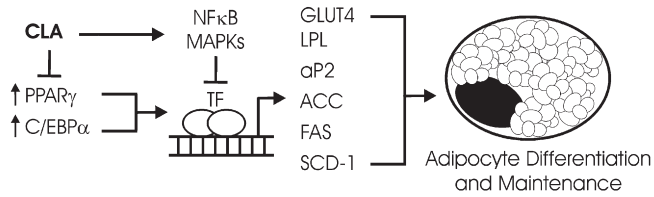

Fig. 1. 10,12-CLA antagonizes the expression and activity of PPARγ and C/EBPα, master regulators of adipocyte differentiation and maintenance. We propose that 10,12-CLA impairs preadipocyte differentiation and maintenance of mature adipocytes by (a) decreasing the expression of PPARγ and C/EBPα, and (b) activating inflammatory proteins such as NFκB and MAPKs that antagonize PPARγ activity, thereby reducing the expression of PPARγ target genes. TF = transcription factor.

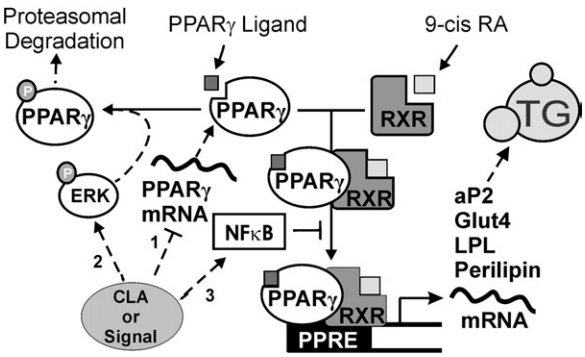

Fig. 2. 10,12-CLA may antagonize PPARγ activity by (1) decreasing PPARγ gene expression; (2) enhancing PPARγ degradation via phosphorylation, ubiquitination and proteosome degradation; or (3) increasing NFκB activation, which impairs PPARγ DNA binding and subsequent induction of adipogenic and lipogenic gene expression.

antiadipogenic effects [88,89], and interleukin (IL) 1β and interferon γ have been observed to induce delipidation of human adipocytes [90]. Treatment with 10,12-CLA has also been shown to increase the expression or secretion of IL-6 and IL-8 from murine [24,50] and human [73,77,86] adipocyte cultures, as well as TNFα and IL-1β, thereby suppressing PPARγ activity and insulin sensitivity [32,76,77,91].

In human subjects, 10,12-CLA supplementation also increases the levels of inflammatory prostaglandins (PGs) [58,92]. For example, women supplemented with mixed CLA isomers (5.5 g/day for 16 weeks) exhibited higher levels of C-reactive protein in serum and 8-*iso*-$PGF_{2\alpha}$ in urine [40]. Accordingly, the expression of cyclooxygenase 2, an enzyme involved in the synthesis of PGs, was elevated in cultures of newly differentiated human adipocytes treated with 10,12-CLA [71]. Furthermore, 10,12-CLA increased $PGF_{2\alpha}$ secretion from human adipocytes [87].

Inflammatory PGs such as $PGF_{2\alpha}$ have been reported to inhibit adipogenesis via phosphorylation of PPARγ by MAPKs [93] and via induction of the normoxic activation of hypoxia-inducible factor-1 (HIF-1). HIF-1 decreases PPARγ and C/EBPα expression by up-regulating the transcriptional repressor DEC1 [94,95]. In addition, $PGF_{2\alpha}$ may inhibit adipogenesis by activating proinflammatory transcription factors that antagonize PPARγ.

Notably, data from our laboratory show that activation of ERK and NFκB plays a critical role in 10,12-CLA's suppression of adipogenic genes and insulin-stimulated glucose uptake [73,86]. The molecular mechanisms by which NFκB and other inflammatory

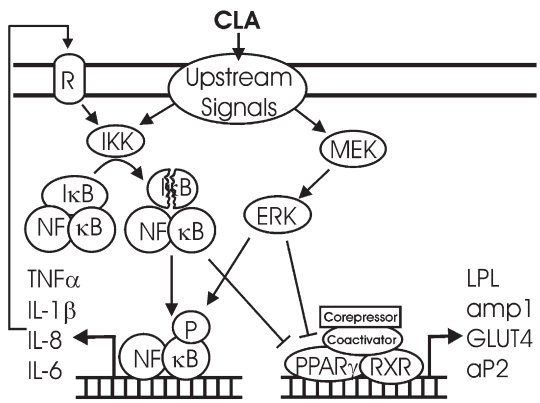

Fig. 3. 10,12-CLA activation of inflammatory proteins and induction of inflammatory genes interfere with PPARγ transcriptional activation of target genes such as *LPL*, adiponectin (*AMP1*), *GLUT4* and *aP2*.

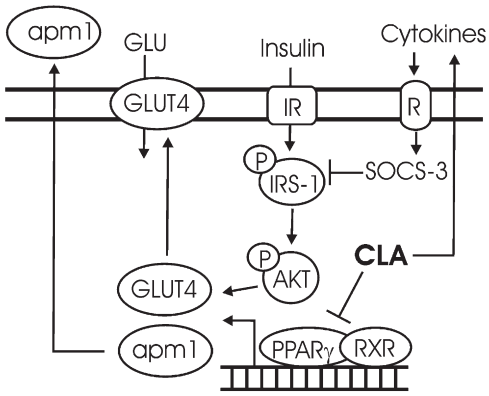

Fig. 4. 10,12-CLA-mediated insulin resistance is linked to (a) antagonism of PPARγ-induced GLUT4 and adiponectin (AMP1) expression, and (b) induction of inflammatory proteins and genes that decrease IRS-1-P (tyr) abundance, thereby reducing GLUT4 translocation to the plasma membrane.

transcription factors inhibit PPARγ activity are not completely understood, but results from a study on the bone marrow stromal cell line ST2 suggest that NFκB interacts directly with PPARγ preventing it from binding DNA [96,97]. In a different study using chromatin immunoprecipitation, the DNA binding activity of PPARγ did not appear to be affected by TNFα stimulation in 3T3-L1 adipocytes or human embryonic kidney 293 cells. Instead, suppression of PPARγ activity involved IKK activation, leading to IκBα degradation and nuclear localization of histone deacetylase 3, a component of the PPARγ corepressor complex [98,99]. NFκB may also repress PPARγ activity via interaction with the DNA-bound retinoid X receptor–PPARγ heterodimer, thereby interfering with coactivator recruitment.

Taken together, these data suggest that 10,12-CLA antagonizes PPARγ activity via inflammatory mediators such as MAPKs and NFκB or via induction of inflammatory PG and adipocytokine production, which in turn antagonize PPARγ activity.

2.4. CLA regulation of lipid metabolism

2.4.1. CLA suppresses lipogenesis

Storage of fatty acids such as triglycerides (TGs) is a major function of adipocytes. Numerous proteins involved in lipogenesis, such as lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD), are decreased by supplementation with mixed isomers of CLA or

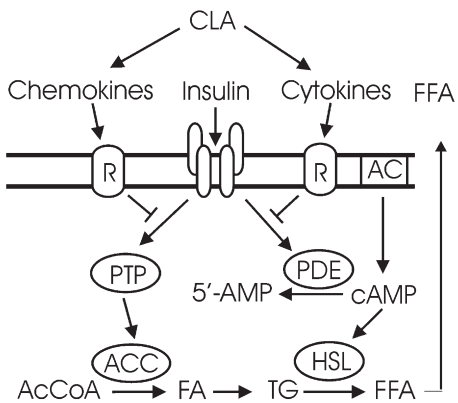

Fig. 5. 10,12-CLA increases lipolysis acutely and decreases lipogenesis chronically by decreasing phosphodiesterase (PDE) and ACC activities, respectively, key proteins regulated by insulin.

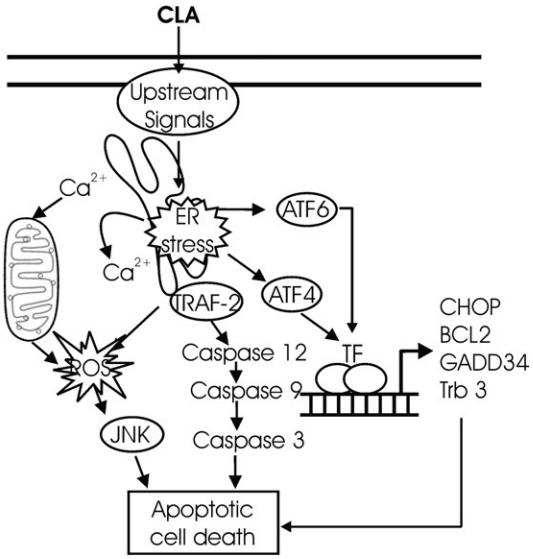

Fig. 6. 10,12-CLA increases apoptotic cell death of (pre)adipocytes by increasing ER stress. 10,12 activates upstream signals that induce cell stress, including ER stress and ISR. These stress responses increase the levels of intracellular calcium, reactive oxygen species and proteins that together induce apoptosis.

10,12-CLA alone [50,72,73,100]. PPARγ is a major activator of many lipogenic genes, including glycerol-3-phosphate dehydrogenase, LPL and lipin, as well as genes encoding lipid-droplet-associated proteins such as perilipin, adipocyte-differentiation-related protein, cell-death-inducing DFFA-like effector c and S3-12 [101]. Thus, 10,12-CLA may exert its antilipogenic effects, in part, through its ability to inhibit PPARγ activity. CLA repression of the lipogenic transcription factor SREBP-1 and its target genes may also play an important role. Finally, CLA suppression of insulin signaling may also affect the activation or abundance of a number of lipogenic proteins, including LPL, ACC, FAS, SCD1 and the insulin-dependent glucose transporter 4 (GLUT4).

Interestingly, 10,12-CLA or a CLA mixture reduce the levels of monounsaturated fatty acids in rodents [27,51] and primary human adipocyte cultures [72]. This may be due to the ability of CLA to repress SCD1 expression [69] and function required for monosaturated fatty acid synthesis [102,103]. However, 10,12-CLA supplementation reduced body weight in SCD1 knockout mice, simultaneously increasing the ratio of 16:0/16:1 fatty acids and decreasing the ratio of 18:0/18:1 fatty acids [49]. These results suggest that the antiobesity properties of CLA may also rely on other desaturases, which may include the isoenzyme SCD2. For instance, body fat loss in mice fed a CLA mixture requires Δ6-desaturase [104].

2.4.2. CLA causes insulin resistance

Insulin-stimulated glucose uptake in WAT is mediated via GLUT4 (Fig. 4). Defect in insulin signaling or suppression of GLUT4 translocation to the plasma membrane is a primary cause of insulin resistance in adipocytes (Figs. 4 and 5). Insulin resistance has been reported in overweight or obese mice [50] or humans [92,105–107] and in cultures of 3T3-L1 [71] or human adipocytes [72,86,87] following supplementation with a CLA mixture or 10,12-CLA alone. Moreover, supplementation with a CLA mixture or 10,12-CLA has been shown to induce hyperinsulinemia, which is associated with insulin resistance in animals and humans (reviewed in Wang and Jones [13]). CLA may inhibit insulin signaling by (a) activating inflammatory pathways and stress kinases and (b) down-regulating the expression of genes involved in the insulin signaling and glucose uptake pathways.

In addition, some studies on 3T3-L1 cells [24] and cultures of newly differentiated human adipocytes [71] have suggested that CLA inhibits insulin signaling via increased expression of suppressor of cytokine signaling 3 (SOCS-3). SOCS-3 impairs insulin signaling and glucose uptake by promoting the phosphorylation of inhibitory serine 307 on insulin receptor substrate 1 (IRS-1), leading to its ubiquitination and proteasome degradation [108]. CLA appears to induce SOCS-3 indirectly via inflammatory cytokines such as TNFα and IL-6 [73,109,110]. 10,12-CLA treatment has also been demonstrated to decrease the protein levels of insulin receptor β (IRβ) [24] and IRS-1 [24,86] — signaling proteins critical for insulin sensitivity. In addition, 10,12-CLA treatment reduced tyrosine phosphorylation (i.e., activation) of IRβ and IRS-1 in 3T3-L1 adipocytes [24].

10,12-CLA may directly impair the uptake of glucose and fructose by suppressing the expression of their transporters. 10,12-CLA decreased GLUT4 gene and protein expression [71,73,86] in cultures of newly differentiated human adipocytes. Similarly, CLA reduced the gene expression of GLUT4 and the glucose/fructose transporter SLC2A5 in WAT and 3T3-L1 adipocytes supplemented with 10,12-CLA [50].

CLA may also cause insulin resistance via its effects on the insulin-sensitizing hormone adiponectin. Adiponectin mRNA levels were decreased following supplementation with 10,12-CLA in mice [24] and in cultures of human adipocytes [73]. Consistent with these data, 10,12-CLA or a CLA mixture decreased adiponectin assembly or secretion in cultures of murine adipocytes, respectively [18,111]. Because adiponectin is a target gene of PPARγ [112], its suppression may be due, in part, to 10,12-CLA-antagonizing PPARγ activity. Accordingly, the PPARγ agonist rosiglitazone was able to prevent CLA-induced suppression of adiponectin serum levels and insulin resistance in mice [94]. However, another PPARγ agonist, troglitazone, did not prevent the 10,12-CLA suppression of adiponectin expression, although it prevented 10,12-CLA suppression of TG levels and adiponectin oligomer assembly in 3T3-L1 adipocytes [18]. These results indicate that CLA may suppress adiponectin expression by a PPARγ-independent mechanism.

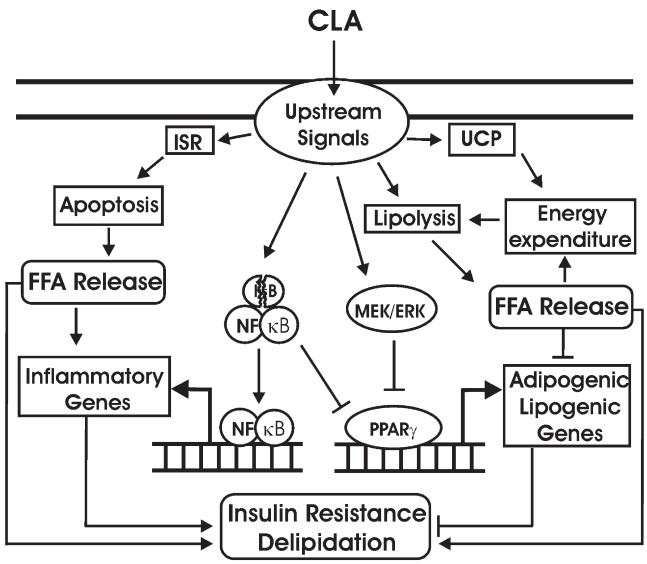

Fig. 7. Working model by which 10,12-CLA causes insulin resistance and delipidation in adipocytes. We propose that 10,12-CLA induces upstream signals that cause (a) an ISR that increases apoptosis, FFA release and inflammatory gene expression; (b) NFκB and ERK activation that antagonizes PPARγ activity; and (c) increased UCP and lipolysis, further enhancing FFA levels. Together, these CLA-mediated signals cause adipocyte insulin resistance and delipidation.

2.4.3. CLA stimulates lipolysis

Lipolysis is the process by which stored TG is mobilized, releasing free fatty acids (FFAs) and glycerol through the action of hormone-sensitive lipase (HSL). Typically, when energy demand is increased, lipolysis is up-regulated via cAMP-mediated signaling. CLA may induce lipolysis in WAT through its activation of proinflammatory pathways, thereby liberating FFA for uptake in metabolically active tissues (i.e., liver and muscle) (Fig. 5). Acute treatment with mixed CLA isomers or 10,12-CLA alone increased lipolysis in 3T3-L1 [19,100,113] and newly differentiated human adipocytes [114]. Furthermore, LaRosa et al. [50] observed increased mRNA levels of HSL in C57BL/6J mice fed 10,12-CLA for 3 days; however, HSL levels subsequently decreased following chronic (17-day) treatment.

Numerous studies in other species have investigated the effect of long-term CLA supplementation on lipolysis. Studies with mice or hamsters have demonstrated that chronic supplementation with a mixture of CLA has no effect on lipolysis [115–117]. In contrast, chronic treatment with CLA (1–200 μmol/L mixed isomers) reduced glycerol release from isolated rat adipocytes [111]. Consistent with these data, FFA levels have been reported to be lower in the serum of OLETF rats supplemented with a CLA mixture [1.0% (wt/wt) for 4 weeks] compared to controls [118]. The lack of a chronic lipolysis effect may be due to depleted TG stores in WAT, which can lead to ectopic lipid accumulation seen in lipodystrophy syndromes. For example, supplementation with a CLA mixture or 10,12-CLA alone increased lipid accumulation in the liver of mice [119,120] and hamsters [121,122]. In summary, CLA induces inflammatory adipokines that likely impair insulin signaling, thereby decreasing TG synthesis and increasing lipolysis, leading to decreased WAT mass (Fig. 5).

2.5. CLA regulation of apoptosis

2.5.1. CLA induces (pre)adipocyte apoptosis

Apoptosis is another mechanism by which CLA may be able to reduce BFM. Studies using mice [33,34,50] or 3T3-L1 murine adipocytes [52,113] supplemented with 10,12-CLA or a CLA mixture have reported apoptosis in adipocytes. For example, mice fed a high-fat diet containing a 1.5% (wt/wt) CLA mixture had an increased ratio of BAX relative to Bcl2, inducer and suppressor of apoptosis in the mitochondrial apoptotic pathway, respectively [76]. Furthermore, supplementation of C57BL/6J mice with a 1% (wt/wt) CLA mixture reduced BFM and increased apoptosis and TNFα gene expression in WAT [123]. TNFα gene expression and secretion have also been reported to be induced in mice by 10,12-CLA alone [16,24]. TNFα is a potent inducer of apoptosis [124] and plays a critical role in adipocyte function [125]. TNFα gene expression was likewise induced by 10,12-CLA in cultures of newly differentiated human adipocytes, although its secretion was not detected [73,91].

Besides the TNFα/death receptor and mitochondrial pathways, apoptosis can occur via activation of integrated stress response (ISR) (Fig. 6). Microarray analysis revealed that 10,12-CLA treatment of mice [1% (wt/wt)] and 3T3-L1 adipocytes (100 μmol/L) increased the mRNA levels of genes involved in ISR such as activating transcription factor 3, C/EBP homologous protein (CHOP), pseudokinase Tribbles 3/SKIP 3 (TRIB3), X-box binding protein, and growth arrest and DNA damage inducible protein (GADD34) [75]. CHOP is known to possess apoptotic characteristics, and activation of this protein can lead to induction of GADD34 and TRIB3 [126,127]. Notably, CLA-induced ISR activation in adipocytes was preceded by the induction of inflammatory genes such as *IL-6* and *IL-8* [75]. In mouse mammary tumor cells, 10,12-CLA treatment (20–40 μmol/L) increased CHOP expression and endoplasmic reticulum (ER) stress, leading to apoptosis [128,129]. Collectively, these data suggest that CLA may induce adipocyte apoptosis via ER stress and ISR, depending on the dose and isomer used. *In vivo* studies are needed to investigate whether the apoptotic effects of CLA on humans are specific for WAT.

3. Conclusion and implications

Supplementation with a mixture of CLA isomers or 10,12-CLA alone reduces adiposity consistently in animal models, especially in rodents, but reduces adiposity in only some human studies. Potential reasons for these species differences include (a) the CLA isomers used, (b) the dosage administered, and (c) the age, body weight, body fat or metabolic status of the animals or subjects. Of the major isomers, only 10,12-CLA reduces the adiposity or TG content of WAT. Dosage differences among species can be considerable; rodent studies generally use ~20 times more CLA per kilogram of body weight than human studies.

Potential mechanisms responsible for these antiobesity properties of 10,12-CLA include (a) decreasing energy intake by suppressing appetite; (b) increasing energy expenditure in WAT, muscle and liver tissue, or LBM; (c) decreasing lipogenesis or adipogenesis; (d) increasing lipolysis or delipidation; and (e) apoptosis via adipocyte stress, inflammation, and/or insulin resistance.

Based on these data, we propose the following working model (Fig. 7) depicting the mechanisms by which 10,12-CLA decreases WAT mass. We speculate that 10,12-CLA binds to a cell surface fatty acid receptor or diffuses/flip-flops into adipocytes, thereby activating upstream signals. These upstream signals induce ISR, FFA release, and activation of NFκB and MAPKs that may directly antagonize PPARγ activity. Increased release of PGs and cytokines may further antagonize PPARγ activity, leading to insulin resistance and delipidation. The resulting FFA accumulation in blood, liver and muscle increases FFA oxidation, and FFA-induced insulin resistance in these tissues. If energy expenditure is not sufficient to completely oxidize these elevated levels of FFAs, hyperlipidemia, hyperglycemia, and lipodystrophy can result.

Future studies are needed to identify potential upstream mediators of this proposed stress cascade in adipocytes. Elucidating these mechanisms will provide valuable information on the efficacy, specificity and potential side effects of CLA isomers as dietary strategies for weight loss or maintenance. Such knowledge is essential for the effective and safe use of CLA supplements to control obesity.

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