Conjugated linoleic acid evokes de-lipidation through the regulation of genes controlling lipid metabolism in adipose and liver tissue

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Summary

Conjugated linoleic acid (CLA) is a unique lipid that elicits dramatic reductions in adiposity in several animal models when included at ≤1% of the diet. Despite a flurry of investigations, the precise mechanisms by which conjugated linoleic acid elicits its dramatic effects in adipose tissue and liver are still largely unknown. *In vivo* and *in vitro* analyses of physiological modifications imparted by conjugated linoleic acid on protein and gene expression suggest that conjugated linoleic acid exerts its de-lipidating effects by modulating energy expenditure, apoptosis, fatty acid oxidation, lipolysis, stromal vascular cell differentiation and lipogenesis. The purpose of this review shall be to examine the recent advances and insights into conjugated linoleic acid's effects on obesity and lipid metabolism, specifically focused on changes in gene expression and physiology of liver and adipose tissue.

Keywords: Adipocyte, adipose tissue, CLA, de-lipidation, lipid metabolism, liver tissue, obesity.

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Conjugated linoleic acid; general information

The conjugated linoleic acid (CLA) family consists of several conjugated and stereoisomeric variations of linoleic acid (*cis, cis*-$\Delta^{9,12}$-octadecadienic acid), of which 16 have currently been identified (1–3). Natural forms of CLA can be found predominantly in ruminant products, such as milk, cheese and beef (3–7), and exist primarily (80–90%) as the *cis*-9, *trans*-11 isomer (c9t11), also known as rumenic acid (7–10). Within ruminants, lamb contains the highest concentration of CLA, and veal the lowest (7). Measurements of c9t11-CLA in human adipose tissue have found that its presence is highly correlated with milk fat intake (11), and CLA intake averages about 200 mg d^{-1} for men and 150 mg d^{-1} for women (12). Anaerobic ruminant bacteria, such as *Butyrivibrio fibrisolvens*, produce CLA (predominantly c9t11-CLA) through biohydrogenation of linoleic acid and α-linolenic acid obtained from plant material (9,13,14). A recent study has shown that endogenous synthesis is responsible for more than 91% of the c9t11-CLA present in milk fat (15). Upon synthesis, CLA is either absorbed or further metabolized to vaccenic acid (*trans*-11-octadecenoic acid) (13), which can be converted back to c9t11-CLA by the enzyme Δ9 desaturase (also called stearoyl-CoA desaturase) (14,16).

Several methods are currently available to chemically synthesize CLA (17–19). Current CLA supplements used in feeding studies are synthesized by alkaline isomerization of linoleic acid-enriched vegetable oils (e.g. safflower and sunflower oil) and are mostly available in a 1:1 ratio of c9t11-CLA and *trans*-10, *cis*-12 (t10c12)-CLA (9,20). Several companies offer dietary supplements containing CLA; examples include Your Life®, Natrol® and Nature's Way® (21). Recently, Loders Croklaan Lipid Nutrition reported they obtained self-affirmed GRAS (generally recognised as safe) status for their product Clarinol™, potentially leading to its use in functional foods in the USA (22). Cogniz, a competing company, expects that their product Tonalin® will receive GRAS status very soon (22). Even though CLA supplements are readily available, specific benefits for

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humans appear to be relatively small and any detrimental effects are subject to further investigation [see Terpstra (20) for detailed review].

The effects of conjugated linoleic acid in the body

In 1987 Ha *et al.* (4) reported that CLA present in fried ground beef reduced tumour incidence in mice chemically treated to induce epidermal neoplasia; their results initiated a flurry of research (visit http://www.wisc.edu/fri/clarefs.htm for a list of publications on CLA since 1987) (14). To date a majority of the experiments using CLA have used an isomeric mix containing approximately a 1 : 1 ratio of c9t11- and t10c12-CLA (approximately 40% and 44%, respectively; referenced as MI-CLA in the remainder of the review) (14). These experiments, both *in vivo* and *in vitro*, have reported that CLA has beneficial effects (at least in some animal species) against cancer [see Belury (23) for review], atherosclerosis [see McLeod (24) for review], diabetes and obesity (reviewed below). Studies also have reported that results vary between species, and that the c9t11- and t10c12-CLA isomers have differential effects on body composition. However, it now appears clear that the t10c12-CLA isomer is responsible for changes *in vivo* and *in vitro*, particularly in body composition and adipocyte morphology (14,25–29). Described below is a brief synopsis of observations in diabetes, followed by a detailed review of CLA's effects on obesity, specifically focused on changes in gene expression and physiology of liver and adipose tissue.

Diabetes

The anti-diabetogenic effects of CLA differ markedly between species *in vivo*, ranging from beneficial effects in rats (30–34) to detrimental effects in mice (28,35–37) and humans (20,38,39). In 1998, Houseknecht *et al.* (30) showed that 1.5% MI-CLA improved hyperinsulinaemia and normalized glucose tolerance in Zucker diabetic fa/fa (ZDF) rats fed for 14 days. They also reported a CLA-mediated increase in adipocyte-specific fatty acid-binding protein 2 (aP2) mRNA levels and activation of the peroxisome proliferator-activated receptor-γ (PPAR-γ) *in vitro*, indicative of modulation through this transcription factor (see below for further review on CLA's interactions with PPAR-γ) (30). Differing *in vitro* results were reported by Granlund *et al.* (40) who recently showed in 3T3-L1 adipocytes that t10c12-CLA did not activate PPAR-γ and selectively inhibited thiazolidinedione-induced PPAR-γ activation, implicating t10c12-CLA as a PPAR-γ antagonist (33). A later *in vivo* study conducted by Ryder *et al.* (31) confirmed CLA's effects on glucose tolerance and insulin action. They reported improved insulin-stimulated glucose tolerance and glycogen synthase activity in soleus muscle of ZDF rats supplemented with MI-CLA, compared with rats fed a control diet or supplemented with c9t11-CLA (91% pure). They further showed that within the 50 : 50 mixture, these effects were predominantly exerted by the t10c12-CLA isomer (31). Similar studies with fa/fa Zucker rats have confirmed these results, and attributed t10c12-CLA's anti-diabetogenic effects to reduced oxidative stress and muscle lipid levels (32,33). A recent *in vivo* study by Nagao *et al.* (34) showed that 1% MI-CLA increased adiponectin gene expression and plasma levels in male ZDF rats fed for 8 weeks. They proposed that this also may be a potential mechanism by which CLA reduces hyperinsulinaemia (34). Several groups report opposite effects *in vivo* in mice (28,35–37) and humans (38,39), showing that t10c12-CLA supplementation leads to insulin resistance. This may result from a decrease in plasma leptin levels (41), or an increase in triacylglycerol levels in muscle by t10c12-CLA (20). The paradoxical effect of the t10c12-CLA isomer in diabetes is a dramatic example of the species-specific differences that exist with supplementation of this fatty acid. However, the degree of obesity in the animal model is an important factor to consider when assessing CLA effects. Indeed, it should be emphasized that a majority of the studies on rats have utilized overtly obese models while murine models were mostly non-obese and that the effects of CLA could vary accordingly. For example, the relative increase in insulin sensitivity observed when obese animals (rats) are fed CLA may result from decreased adiposity, whereas the effect in lean (murine) models may stem from the lipodystrophic effects of CLA.

Obesity

The de-lipidative effects of MI-CLA were first observed by Park *et al.* (42) in the ICR (Institute for Cancer Research) line of mice supplemented with 0.5% CLA. They reported a 60% decrease in body fat after about 4–5 weeks of feeding (42). Similarly, studies using MI-CLA and predominantly the t10c12-CLA isomer reported decreased body fat in other lines of mice [see Pariza *et al.* (14) for review]. Reductions in adiposity have been reported in Sprague-Dawley and Zucker (lean) rats; however, the effects are not as striking as in mice (25–30%) (43–45). Interestingly, MI-CLA increased fat deposition in obese and albino rats (45,46). Sisk *et al.* (45) showed that 0.5% MI-CLA reduced insulin levels in male and female obese Zucker rats fed for 5 and 8 weeks, respectively, and suggested that the increased fat could be attributed to a normalized glucose tolerance, coupled with hyperphagia. A recent study conducted with rats fed for 28 days varied the source of protein (either casein or soy) in 0, 0.1, 0.5 and 1.0% MI-CLA-supplemented diets (47). The results showed that the de-lipidative effect of CLA was more pronounced in rats

fed the soy diet, indicating that dietary protein may alter the effects of CLA and contribute to differing observations between experiments (47). In swine MI-CLA decreased fat deposition and increased lean tissue (48–52). Generally, experiments conducted in humans have shown that neither MI-, c9t11- or t10c12-CLA has a significant effect on body weight (20,21,53). In a randomized double-blind trial using 60 overweight or obese people supplemented for 12 weeks, Blankson *et al.* (54) showed that MI-CLA reduced body fat mass but had no effect on body mass index (BMI) (54). Similar effects have been reported in other human trials with MI- (55–57) and t10c12-CLA (57). It should be noted that the loss of body fat is 40–50% greater in mice than observed in humans (20); however, mice are generally fed approximately five times more CLA than humans (per kilogram body weight). In summary, de-lipidative effects of MI- and t10c12-CLA have been demonstrated in mice (42), rats (43–45,47), pigs (48–52) and humans (note that CLA reduced body fat mass but had no effect on BMI) (54–57). In contrast, negative effects of CLA on adiposity have been shown in obese rats (45,46), but not in humans (20,21,53).

Feed intake

An issue that has remained controversial is the effect of CLA on feed intake. Several studies in mice, rats and pigs have reported that CLA has little to no effect on feed intake (36,43–45,49–51,58–60), while others have reported a reduction in feed intake (27,31,46,48,52,61–63). However, studies conducted in mice and rats with a pair-fed group on a CLA-supplemented diet confirmed a significant decrease in fat pad mass compared with pair-fed controls, indicating that a reduction in feed intake does not solely account for a reduction in fat mass (27,31,64). It is possible that CLA either has an adverse organoleptic quality, or alters metabolism in such a way as to impart a reduction in feed intake. Given the phenotypic effects on metabolic tissues (e.g. adipose and liver) reported in the literature, the latter possibility is most probable; however, confirmation hinges upon further investigation.

Lipid metabolism

Several *in vivo* studies in mice and rats have shown that CLA incorporates into membrane phospholipids and alters fatty acid homeostasis (65–70), with c9t11-CLA accumulating to a greater extent than t10c12-CLA in liver phospholipids (65). However, Brown *et al.* (71) showed that *in vitro*, t10c12-CLA readily incorporated into the neutral and phospholipid fractions of human preadipocyte cultures exposed for 12 d. Upon absorption, CLA that is not catabolized through β-oxidation by hepatocytes in mice (66) and rats [*in vivo* (68) and *in vitro* (72,73)] is converted into a conjugated 18:3 product by Δ6 desaturase and then further elongated and desaturated into conjugated 20:3 and 20:4. More specifically, Sebedio *et al.* (68) showed that in rats supplemented with either the c9t11- or t10c12-CLA isomer, the t10c12-CLA isomer was preferentially metabolized to 16:2 and 18:3 conjugated isomers and c9t11-CLA to a conjugated 20:3 isomer. The physiological effects of CLA may partly result from competition with linoleate as substrate for Δ6 desaturation. This is the rate-limiting step for arachidonate formation from linoleate, and implicates CLA [specifically t10c12-CLA *in vitro* in human preadipocytes (71)] in reduced arachidonate accumulation in phospholipids and subsequently reduced eicosanoid production *in vivo* in mice and *in vitro* in human preadipocytes (65,66,71).

In addition to adipose tissue, a major organ affected by CLA treatment is the liver. When mice are supplemented with CLA, the liver becomes steatotic and increases in mass up to four times, an effect exerted predominantly by the t10c12-CLA isomer (28,35,66,69,74). A CLA study in mice attributed this to an increase in liver triglycerides (TGs), cholesterol, cholesterol esters and FFAs (69); opposite effects on liver TGs have been reported in the rat (68). The t10c12-CLA isomer also was associated with an increase in 18:1 n-9 and a decrease in 18:2 n-6 (69) and has been shown to alter fatty acid profiles in rats and pigs (67,68,75). Other experiments conducted *in vivo* in rats (68), mice (76) and pigs (77), and *in vitro* with human preadipocytes (71), HepG2 cells (78) and 3T3-L1 adipocytes (79), have confirmed an increase in the ratio of saturated fatty acids to monounsaturated fatty acids (SFA : MUFA), in particular palmitate : palmitoleate (16:0/16:1) and stearate : oleate (18:0/18:1) with t10c12-CLA supplementation [for a review on effects between species, see Evans *et al.* (80)]. This is likely caused by a reduction in stearoyl-CoA desaturase-1 (discussed later in this review), an enzyme that catalyses the biosynthesis of monounsaturated fatty acids preferentially from 16:0 and 18:0 substrates (76,81). The early effects of t10c12-CLA in mice had no impact on the SFA : MUFA ratio after 4 days of supplementation (82), indicating that the change might be indirect instead of a direct effect of CLA.

How does conjugated linoleic acid work? Mechanistic elucidation

Currently, mechanisms by which CLA imparts its dramatic effects on liver and adipose tissue are largely unknown. A review of the literature indicates that it reduces adiposity by increasing energy expenditure, apoptosis, fatty acid oxidation, lipolysis and inflammation, as well as decreasing energy intake, stromal vascular (SV) cell differentiation and lipogenesis (Fig. 1). In an effort to further elucidate the de-lipidative mechanisms of CLA, several groups have conducted experiments analysing protein and gene expression of molecules involved in metabolism of the liver and adi-

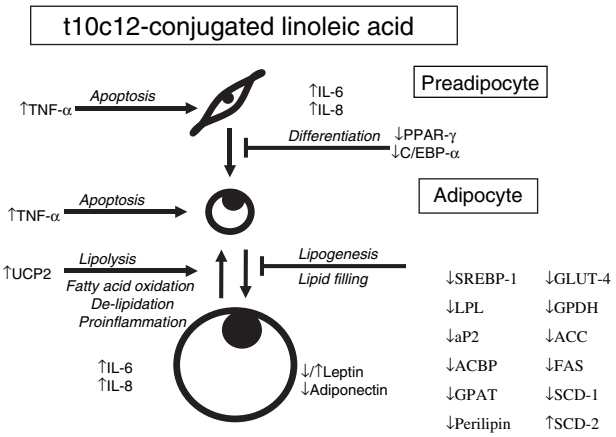

Figure 1 Proposed model of the de-lipidative effects of t10c12-CLA on mRNA concentration, depicting the differentiation of a preadipocyte to an adipocyte and subsequent lipid filling of the adipocyte. The model suggests that conjugated linoleic acid (CLA) imparts its effects by increasing expression of genes associated with apoptosis, fatty acid oxidation, lipolysis and inflammation, as well as decreasing stromal vascular cell differentiation, and lipogenesis (as indicated by horizontal or blocked arrows). Expressions of specific genes modulated (↑ or ↓) by t10c12-CLA are annotated adjacent to their respective function.
IL, interleukin; SREBP-1, sterol regulatory element-binding protein-1; LPL, lipoprotein lipase; aP2, fatty acid-binding protein 2; ACBP, acyl-CoA-binding protein; GPAT, glycerol-3-phosphate acyltransferase; GLUT-4, glucose transporter-4; GPDH, glycerol-3-phosphate dehydrogenase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase; TNF-α, tumour necrosis factor-α; UCP, uncoupling protein; PPAR-γ, peroxisome proliferator-activated receptor-γ; C/EBP-α, CCAAT/enhancer-binding protein α.

pose tissue. A summary of this literature with respect to gene expression is provided in Table 1.

Adipose tissue

One of the mechanisms by which CLA imparts its effects is by increasing energy expenditure (shown with MI-CLA supplementation) (59–62). Several groups have offered insight into mechanisms by which energy is expended with CLA treatment. Recently the uncoupling proteins have been of particular interest in energy expenditure and oxidation. Uncoupling proteins (UCPs) are a family of several molecules that include UCP1, UCP2 and UCP3 (100). Predominantly expressed in the mitochondrial inner membrane of brown adipose tissue, UCP1 forms a proton channel that leaks protons that would otherwise be used to drive adenosine triphosphate (ATP) synthesis, resulting in the production of heat (101). Precise functions of UCP2 and UCP3 are currently unclear, but they have been implicated in regulation of insulin secretion and fatty acid metabolism, respectively (100,102); UCP2 is generally expressed ubiquitously, but is abundantly expressed in white adipose tissue, and UCP3 is predominantly expressed in skeletal muscle (103). Upon CLA supplementation, several *in vivo* studies have reported an increase in UCP2 expression (an effect which is most prominent with t10c12-CLA supplementation) (31,35,60,81,83,84), in brown and white adipose tissue and either no effect (60) or a decrease in UCP1 and UCP3 expression (83,84).

In addition to increasing energy expenditure, t10c12-CLA reduces adipose tissue mass by initiating apoptosis and modulating differentiation of preadipocytes. Using a polygenic obese line of mice (M16), Miner *et al.* (63) showed that apoptosis in retroperitoneal fat pads of mice fed MI-CLA was fourfold greater than observed in control mice. They reported an approximately 50% decrease in the weight of retroperitoneal fat pads and an approximately 40% decrease in the weight of epididymal fat pads after 5 days of treatment with MI-CLA (63). In later work, Hargrave *et al.* (27) confirmed *in vivo* that the t10c12-CLA isomer was predominantly responsible for this effect. Interestingly, they also reported that the reduced fat effect of t10c12-CLA was independent of genetic strain (excluding the effect on adipocyte apoptosis) (27). Evans *et al.* showed that 3T3-L1 adipocytes treated with 100 μM MI-CLA (major effect was shown to be from t10c12-CLA) showed increased apoptotic activity (104). However, 30 μM t10c12-CLA did not appear to cause apoptosis in cultures of human adipocytes (71,86). Studies *in vivo* with mice have shown that an increase in the tumour necrosis factor-α (TNF-α) mRNA levels [a cytokine shown to induce leptin production, lipolysis, adipocyte de-differentiation, insulin resistance, as well as apoptosis of pre- and mature adipocytes (105)] in adipose tissue resulted from MI-CLA supplementation, further indicating that apoptosis is one of the probable mechanisms of murine de-lipidation exerted by CLA (35,85). However, in an *in vitro* analysis with human adipocytes, Brown *et al.* found that t10c12-CLA had no effect on TNF-α (86). It therefore appears that with respect to adipocyte apoptosis, the CLA effect *in vitro* (human adipocytes) contrasts with effects *in vivo* (mice).

Trans-10, *cis*-12-conjugated linoleic acid also inhibits preadipocyte proliferation and differentiation *in vitro* (104,106,107). While at first there was some conflict (79), it now seems that modulation of preadipocyte differentiation by t10c12-CLA appears to be driven, in part, by down-regulation of PPAR-γ expression (108). An exception is CLA's interactions with porcine adipocytes *in vitro*, which have shown either an opposite or no effect on PPAR-γ (89,109). After reporting an increase in porcine preadipocyte differentiation and no increase in PPAR-γ mRNA concentration in porcine SV cells exposed to either t10c12-, c9t11- or MI-CLA for 2 days, McNeel and Mersmann (89) suggested that the differing effects of CLA depended on species, CLA isomer and experimental conditions. It should be noted that after 2 days of CLA (c9t11-, t10c12-, MI-CLA) treatment, they observed no further

Table 1 List of genes in adipose and liver tissue effected by conjugated linoleic acid (CLA). Genes are classified by their ascribed function and in juxtaposition to each is the type of isomer used in the studies, the reported effect and species it was observed in

Gene	Isomer	Effect	Species	Study	Literature
Adipose					
Energy expenditure					
Uncoupling protein-1*	MI	↓	Mouse	*In vivo*	(83,84)
		↔	Rat		(60)
					(31,83)
Uncoupling protein-2†	MI t10c12	↑	Mouse	*In vivo*	(31,35,60,81,83,84)
	MI	↔	Rat		(83)
Uncoupling protein-3	MI	↓	Mouse	*In vivo*	(83,84)
		↔	Rat		(83)
Apoptosis					
Tumor necrosis factor-α	MI	↑	Mouse	*In vivo*	(35,85)
	t10c12	↔	Human	*In vitro*	(86)
Differentiation					
Peroxisome proliferator-activated receptor-γ	MI t10c12	↓	Mouse	*In vivo*	(35,81,84)
				In vitro	(40,87,88)
	t10c12	↔	Human		(40,71,86)
			Porcine		(89)
CAAT/enhancer-binding protein-α	t10c12	↓	Human	*In vitro*	(71)
Fatty acid-binding protein 2 (aP2)	t10c12	↓	Mouse	*In vitro*	(40,88)
			Human		(40,71)
	MI	↑	Rat	*In vivo*	(30)
Acyl-CoA-binding protein	t10c12	↓	Human	*In vitro*	(71)
Perilipin	t10c12	↓	Human	*In vitro*	(71)
Adipokines					
Leptin	MI t10c12	↑	Mouse	*In vivo*	(84,90)
	t10c12		Rat		(91)
		↓	Human	*In vitro*	(71)
Adiponectin	t10c12	↓	Mouse	*In vivo*	(90)
Inflammation					
Interleukin-6	t10c12	↑	Human	*In vitro*	(86)
Interleukin-8	t10c12	↑	Human	*In vitro*	(86)
Fatty acid/glucose transport					
Lipoprotein lipase	MI t10c12	↓	Mouse	*In vivo*	(25,42,82)
				In vitro	(88,92)
		↔	Porcine		(89)
Glucose transporter 4	MI	↓	Mouse	*In vivo*	(84)
	t10c12		Human	*In vitro*	(71)
Lipolysis					
Hormone-sensitive lipase	t10c12	↓	Human	*In vitro*	(71)
Lipogenesis					
Acetyl-CoA carboxylase†	MI	↓	Mouse	*In vivo*	(35,85,93)
	t10c12		Human	*In vitro*	(71)
			Bovine		(94)
Fatty acid synthase†	MI	↓	Mouse	*In vivo*	(35,93)
	t10c12			*In vitro*	(88)
			Bovine		(94)
Stearoyl-CoA desaturase 1†	t10c12	↓	Mouse	*In vitro*	(79)
				In vivo	(93)
			Bovine	*In vitro*	(94)
Stearoyl-CoA desaturase 2	t10c12	↑	Mouse	*In vitro*	(88)
Glycerol-3-phosphate dehydrogenase	t10c12	↓	Human	*In vitro*	(71)
Glycerol-3-phosphate acyltransferase‡	t10c12	↓	Bovine	*In vivo*	(95)
Sterol regulatory element-binding protein-1	MI	↓	Mouse	*In vivo*	(35)

Table 1 *Continued*

Gene	Isomer	Effect	Species	Study	Literature
Liver					
Fatty acid synthesis					
Acetyl-CoA carboxylase	MI	↑	Mouse	*In vivo*	(96)
	t10c12	↔			(93)
Fatty acid synthase	MI	↑	Mouse	*In vivo*	(96)
	t10c12	↔			(93)
ATP-citrate lyase	MI	↑	Mouse	*In vivo*	(96)
Malic enzyme	MI	↑	Mouse	*In vivo*	(96)
Stearoyl-CoA Desaturase 1	t10c12	↔	Mouse	*In vivo*	(93)
Fatty acid oxidation					
Carnitine palmitoyl-transferase I (liver)	MI t10c12	↑	Mouse	*In vivo*	(96,97)
Carnitine palmitoyl-transferase I (muscle)	t10c12	↑	Mouse	*In vivo*	(97)
Carnitine palmitoyl-transferase II	MI	↑	Mouse	*In vivo*	(96)
Trifunctional enzyme-α	MI	↑	Mouse	*In vivo*	(96)
Trifunctional enzyme-β	MI	↑	Mouse	*In vivo*	(96)
Peroxisomal acyl-CoA oxidase	MI t10c12	↑	Mouse	*In vivo*	(90,96–98)
Peroxisomal bifunctional enzyme	MI	↑	Mouse	*In vivo*	(96)
Peroxisome proliferator activated receptor-α	t10c12	↓	Mouse	*In vivo*	(90)
Fatty acid-binding protein	MI	↑	Rat	*In vitro*	(99)
Cytochrome P450	t10c12	↑	Mouse	*In vivo*	(90)
	MI	↔	Rat	*In vitro*	(99)

ATP, adenosine triphosphate; MI, mixed isomer-CLA; t10c12, *trans*-10, *cis*-12-CLA; ↑, ↓, ↔; mRNA concentration increase, decrease or no effect, respectively.

*Observed in brown adipose tissue.

†Observed in mammary tissue as well as in adipose.

‡Observed in mammary tissue.

change in porcine SV cell differentiation or PPAR-γ mRNA concentration.

Both *in vivo* studies in mice (35,81,84) and *in vitro* studies in human (71,86), and 3T3-L1 adipocytes (40,87,88), have confirmed that, upon CLA [predominantly t10c12-CLA (40,71,88)] supplementation, there is a decrease in PPAR-γ expression, indicating a reduction in differentiation of preadipocytes to mature adipocytes. Additionally in these species, there is a decrease in the mRNA levels of CCAAT/enhancer-binding protein α (C/EBP-α) and PPAR-γ target genes such as aP2, perilipin-A and acyl-CoA-binding protein (ACBP) (40,71,88). In contrast, *in vivo* studies with rats show activation of PPAR-γ and an increase in aP2 expression (observed with MI-CLA supplementation) (30,110). Interestingly, Brown *et al.* (71) observed a t10c12-CLA-mediated increase in leptin gene expression *in vitro* with human adipocytes. This finding is in contrast to *in vivo* studies that have reported a decrease in leptin mRNA levels in mice and rats (84,90,91), and no effect or a decrease in serum leptin levels in humans and rats, respectively (38,43,111,112). However, Brown *et al.* (86) confirmed their previous observations *in vitro* on t10c12-CLA-mediated increase in leptin gene expression and demonstrated that t10c12-CLA increased production and secretion of the proinflammatory adipokines interleukin-6 (IL-6) and IL-8 from human SV cells containing newly differentiated adipocytes (86). They proposed a mechanism where, upon secretion from SV cells, IL-6 and IL-8 exert paracrine effects on neighbouring newly differentiated adipocytes (86). This may lead to phosphorylation of transcription factors through induction of mitogen-activated protein kinase kinase/extracellular signal-related kinase (MEK/ERK) signalling that in turn inhibits expression of PPAR-γ and its downstream targets (86). This would then lead to a decrease in glucose and fatty acid uptake and their subsequent synthesis into TG (86).

In agreement with observations on t10c12-CLA-mediated decrease in glucose and fatty acid uptake, several *in vivo* and *in vitro* studies have reported a reduction in the expression of lipoprotein lipase (LPL: fatty acid uptake) (25,42,71,82,86,88,92) and glucose transporter-4 (GLUT-4) (71,84,86). Other studies have analysed the effects of CLA on energy metabolism in adipose tissue, showing that t10c12-CLA increased fatty acid oxidation in 3T3-L1 adipocytes, thereby suggesting another mechanism by which it lowers TG content (26,42). Similar effects also have been reported *in vivo* with rats (113). Associated with these observations, there is a MI-CLA-mediated increase in car-

nitine palmitoyl transferase (CPT) (a mitochondrial membrane bound protein essential for shuttling long chain fatty acids into mitochondria for β-oxidation) activity *in vivo* in mice and rats (42,114). In contrast, Brown *et al.* (86) reported a decrease in fatty acid oxidation *in vitro* in human adipocytes treated with t10c12-CLA, but not c9t11-CLA. With exception of UCPs, at the time of this writing, there have been no studies analysing differential gene expression response to CLA associated with fatty acid oxidation in adipose tissue.

Currently, effects of CLA *in vivo* and *in vitro* on lipolysis are conflicting. Several *in vitro* studies have reported an increase in lipolysis in response to t10c12- or MI-CLA (42,71), while no effect has been reported *in vivo* in mice (82). Interestingly, Brown *et al.* (71) reported that a 9-day treatment *in vitro* with t10c12-CLA decreased the expression of hormone-sensitive lipase, a key enzyme in the lipolytic cycle, suggesting a possible reduction in lipid breakdown. Therefore, it should be noted that effects of CLA on gene expression may not accurately correspond to protein activity, or CLA's effects on lipolysis are time dependent.

In association with a decrease in LPL activity, several *in vivo* and *in vitro* studies have reported that t10c12-CLA decreases lipogenesis (26,41,71,86,115). Two studies [*in vivo* in mice (82) and *in vitro* with human adipocytes (115)] attribute the predominant t10c12-CLA de-lipidative effect to lipogenesis inhibition, especially in early response to CLA (4 days) supplementation, instead of increased lipolysis (82,115). Lin *et al.* (93) recently reported that t10c12-CLA was a more potent inhibitor of *de novo* lipogenesis than c9t11-CLA in the mammary gland of lactating mice. At the gene expression level, both *in vivo* and *in vitro*, there is a reported reduction in acetyl-CoA carboxylase (ACC) (35,71,93,94), fatty acid synthase (FAS) (35,88,93,94) and stearoyl-CoA desaturase (SCD)-1 (in mammary tissue) (93,94), as well as the glycolytic/lipogenic enzyme glycerol dehydrogenase (*in vitro*) (71) following treatment with t10c12-CLA. Interestingly, Kang *et al.* (81) reported an increase in SCD-2 expression in adipose tissue, with a decrease in 18:0/18:1 in mice treated with t10c12-CLA for 4 weeks. Milk fat depression in dairy cattle resulted in increased levels of endogenous t10c12-CLA in milk fat, which was correlated with a reduction in mRNA levels of ACC, FAS, LPL and glycerol phosphate acyltransferase (95). Tsuboyama-Kasaoka *et al.* (35) also reported a decrease in sterol regulatory element-binding protein-1 (SREBP-1) expression *in vivo*. The SREBPs represent a family of transcription factors that include SREBP-1a, SREBP-1c and SREBP-2. Collectively, SREBPs are involved in transcriptional activation of more than 30 genes associated with cholesterol, fatty acid, TG and phospholipid synthesis (116). SREBP-1a can activate all of the SREBP-responsive genes, and SREBP-1c is more specifically associated with fatty acid synthesis, as is SREBP-2 with cholesterol synthesis (116). Once cleaved by proteolysis, SREBP-1 releases a fragment that translocates to the nucleus and activates transcription (94). Treatment of a bovine mammary cell line (MAC-T) with t10c12-CLA did not yield a reduction in SREBP-1 mRNA concentration; however, there was a reduction of the SREBP-1 nuclear fragment, indicating that t10c12-CLA may reduce lipogenesis and lipogenic gene mRNA concentration by inhibiting the proteolytic cleavage of SREBP-1 (94).

Taken together, these data suggest that t10c12-CLA imparts its de-lipidative activity through both metabolism and cell cycle control. Further research will be necessary to elucidate the basis for differences between species and confirm *in vitro* observations *in vivo*.

Liver

Currently, mechanisms by which the liver becomes steatotic in response to CLA are unknown. Several *in vivo* and *in vitro* studies have reported an increase in both liver fatty acid synthesis and oxidation in response to MI-CLA supplementation (98,99,117). Takahashi *et al.* (96) confirmed these observations *in vivo* at both the gene and protein level in two lines of mice (ICR and C57BL/6J); however, the degree of increase resulting from MI-CLA supplementation differed between lines for some enzymes. For fatty acid synthesis, they reported an increase in activity and mRNA levels of ACC, FAS, ATP-citrate lyase and malic enzyme (96). Tsuboyama-Kasaoka *et al.* (85) also reported an increase in hepatic ACC mRNA level *in vivo*, but not FAS. For fatty acid oxidation, Takahashi *et al.* (96) reported an increase in hepatic activity of mitochondrial and peroxisomal palmitoyl-CoA oxidation, CPT, peroxisomal acyl CoA oxidase (ACO), 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase, coupled with an increase in mRNA level of CPT I and II, trifunctional enzyme-α and -β, ACO and bifunctional enzyme *in vivo*. Several other *in vivo* experiments in mice also reported that t10c12-CLA causes an increase in CPT activity, with a greater effect being exerted by the t10c12-CLA isomer (97,118). Degrace *et al.* (97) recently described an increase in CPT I activity in mice supplemented with t10c12-CLA, along with an increased rate of carnitine-dependent palmitate oxidation. They also reported that t10c12-CLA increased the expression of the CPT I liver isoform, CPT I muscle isoform, and CPT II by 100, 200 and 200%, respectively, compared with controls (97). This was accompanied by an increase in ACO activity and gene expression (97). Similar CLA-mediated increases in ACO gene expression have been previously reported *in vivo* with mice (90,98). Degrace *et al.* (97) also observed a t10c12-CLA-induced increase in mitochondrial and peroxisomal fatty acid oxidation capacity when measured *in*

vitro, and suggested that hepatic steatosis is not a result of decreased oxidation.

Because PPAR-α has been shown to regulate expression of enzymes associated with hepatic oxidation (117,119), CLA may mediate its effects through this transcription factor; indeed both c9t11- and t10c12-CLA isomers are ligands of PPAR-α and PPAR-β/δ (28,99). In support of this concept, both CLA isomers (c9t11- and t10c12-) increase expression of cytochrome P450A1 (CYP4A1: ω-hydroxylation of fatty acids), ACO and liver fatty acid-binding protein *in vivo* in mice (90) and *in vitro* in rats (99), all three of which are known gene targets of PPAR-α regulation. However, a study conducted by Peters *et al*. (117) in PPAR-α null mice showed that genes coding liver fatty acid oxidation and fatty acid binding were still affected by CLA, and were therefore modulated independent of PPAR-α. This may result from possible interactions between CLA and PPAR-β/δ which may serve as a PPAR-α-independent mediator in response to CLA supplementation (117). Additionally, Warren *et al*. (90) reported a decrease in PPAR-α expression *in vivo* with mice by t10c12-CLA, while still observing an increase in ACO mRNA levels. In contrast, PPAR-α expression was increased by c9t11-CLA. Therefore, it is probable that the effect of t10c12-CLA is not solely dependent on PPAR-α.

Effects of t10c12-CLA on the SFA : MUFA ratio may result from a decrease in SCD-1 expression (71,76,78,79). However, a recent study using SCD-1 null mice showed that t10c12-CLA exerts its de-lipidative effects independent of SCD-1 (81). Interestingly, SCD-1 null mice had a heavier liver mass, but displayed reduced hepatomegaly compared with wild type (81). Supplementation with t10c12-CLA significantly reduced adipose tissue mass, but did not significantly increase fat accumulation in liver or muscle, potentially confirming *in vivo* observations in earlier experiments that CLA increases energy expenditure (59–61,81). In summary, use of knockout mice has proven to be a valuable tool in clarifying proposed CLA mechanisms. These studies suggest that CLA's impact on liver lipid metabolism does not solely result from its effects on PPAR-α or SCD-1, indicating that it exerts its effects via multiple mechanisms.

Conclusion

It is becoming increasingly clear that the t10c12-CLA isomer of CLA is responsible for many of the effects seen in diabetes and obesity (14,25–29,31–33,40). The fat-reducing effects of CLA have been replicated in several species, with the most dramatic effects observed in mice (42), and thus the murine models have been the object of intense research. Despite this investigative flurry, the precise mechanisms by which t10c12-CLA elicits its dramatic effects in adipose and liver tissue are still largely unknown. While a reduction in feed intake, as reported in some experiments (27,31,46,48,52,61–63), may account for some decrease in adipose tissue mass, pair-feeding trials have established that it is not solely responsible for this effect (27,31,64). Indeed, given the dramatic effects of t10c12-CLA, it is probable that diminution in energy intake may itself be a result of perturbations on metabolic homeostasis, rather than some organoleptic quality of the molecule.

In vivo and *in vitro* analyses of physiological modifications imparted by CLA on protein and gene expression have suggested that CLA exerts its de-lipidating effects by modulating energy expenditure, apoptosis, fatty acid oxidation, lipolysis, inflammation, SV cell differentiation and lipogenesis (Fig. 1). We have reviewed the effect of CLA on genes associated with these processes in different species (Table 1). While the promise of CLA as a therapeutic agent for obesity is currently unclear, the potential for its use as a research tool to elicit a rapid and pronounced reduction in adipose tissue, as well as mechanistic elucidation of how it imparts this effect, will continue to add invaluably to the growing understanding of genetically induced obesity, as well as dietary and pharmacological methods for its treatment.

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