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RESEARCH REVIEW

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Bioactivities and Potential Mechanisms of Action for Conjugated Fatty Acids

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Abstract Since conjugated linoleic acid (CLA) was identified as a principal anticancer component from ground beef in the 1980s, CLA research has discovered that CLA has a wide range of biologically beneficial effects. Clinical studies with CLA are on the rise, and it is apparent that CLA may not be as effective in humans as in rodents, in particular its anti-obesity aspect. In addition, research with regard to other conjugated fatty acids as well as CLA metabolites is still in its infancy. Investigation of bioactivities for other conjugated fatty acids and CLA metabolites may help to extend the understanding of CLA and its mechanisms of actions. This may pose an opportunity to use CLA more efficiently and expand the future use of other conjugated fatty acids as pharmacological agents to assist current treatments.

Keywords: conjugated linoleic acid (CLA), conjugated *trans* fatty acid, obesity, cardiovascular diseases, cancer, bone

Introduction

Current health concern over *trans* fats is particularly linked from partially hydrogenated vegetable oils (1,2). This group of *trans* fats is distinguished as 'non-conjugated *trans* fatty acids' from 'conjugated *trans* fatty acids', which is mainly conjugated linoleic acid (CLA). CLA was originally found as an anticancer component from ground beef in the 1980s (3,4). Since then a number of biologically beneficial effects of CLA have been identified; inhibition of carcinogenesis in several animal models, reduction of severity of atherosclerosis, reduction of adverse effects of immune stimulation, promotion of growth in young rats, and reduction of body fat and an increase in lean body mass in several animal species (5).

Origins of CLA

The primary CLA isomer that occurs in natural products (80-90%), such as beef, milk, and dairy products, is the *cis*-9,*trans*-11 isomer (6). This isomer originates from biohydrogenation of linoleic acid to stearic acid by rumen bacteria (7). Alternatively, *trans*-11 vaccenic acid, which is the primary isomer for ruminant *trans* fatty acids, can be converted to the *cis*-9,*trans*-11 CLA by delta-9 desaturation in mammalian tissues (8,9). In addition to the *cis*-9,*trans*-11 isomer, CLA synthetically prepared from linoleic acid by alkali isomerization contains the *trans*-10,*cis*-12 CLA isomer in about the same level as the *cis*-9,*trans*-11 isomer. Even though the *trans*-10,*cis*-12 CLA isomer is naturally present in food as a minor component, this isomer is considered to be 'man-made' (6,10,11).

Besides those 2 isomers, a number of CLA isomers are found in natural food sources, with double bonds at [7,9], [8,10], [9,11], [10,12], [11,13], and [12,14] (12,13). Among these minor isomers, the anti-platelet aggregation and an antiproliferative effect of the *trans*-9,*trans*-11 CLA isomer and potential modification of cholesterol metabolism of the *trans*-8,*cis*-10 CLA isomer are reported (14-17). Currently, most CLA research reports on the activities of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers, and this review will primarily focus on these 2 main isomers.

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Biological Activities of CLA

Anticancer effects of CLA As the original discovery of CLA was as an anticancer component, CLA has proven to be an effective prevention tool in a number of animal cancer models, such as skin, forestomach, colon, mammary, lung, prostate, and liver (18-21). It has been suggested that CLA can be involved in all stages of cancer; initiation, promotion, progression, and metastasis of cancer (18-20,22). As for its anticancer mechanisms, it is suggested that CLA in part reduces cancer through reducing eicosanoids production, interfering with cell signaling pathways, inhibiting DNA synthesis, enhancing apoptosis, and inhibiting angiogenesis (18-20,23). Both isomers have proven to be effective in prevention of cancer, while others reported differences in anti-cancer activities between these isomers (22,24-27).

Prevention of cardiovascular diseases CLA has been reported to reduce atherosclerotic lesions in several animal models (28-30). CLA reduced total cholesterol, triacylglycerides (TG), and low-density lipoprotein (LDL)-cholesterol and increased high-density lipoprotein (HDL)-cholesterol in animal models (20,31,32). CLA reduces the risk of atherosclerosis thru reduced blood pressure, or involvement of peroxisome proliferator-activated receptor (PPAR, key for lipogenesis), sterol regulatory element-binding proteins (SREBPs, key for fatty acid synthesis and elongation), steroyl-CoA desaturase (SCD, key for TG and cholesterol formation), acetylated LDL, acyl-coenzyme A:cholesterol acyltransferase, and/or cholesteryl ester hydrolase (20,33-37). It has been suggested that the *trans*-

10,*cis*-12 CLA isomer is responsible for these effects (37-39).

Body fat reduction by CLA One of most interesting activities of CLA is its ability to reduce body fat in animals (5). It has been confirmed that the *trans*-10,*cis*-12 isomer is the isomer responsible for this activity, while the *cis*-9,*trans*-11 CLA isomer has no effect on body composition (5,40). CLA's effect on body fat reduction is suggested to be the result of multiple mechanisms: increasing energy expenditure, reducing lipid accumulation in adipose tissues, and/or adipocytes differentiation, increasing adipocyte apoptosis, modulating adipokines and cytokines, such as leptin, tumor necrosis factor-α (TNF-α), adiponectin, or interleukins, and increasing fatty acid β-oxidation in skeletal muscle (5). However, it was recently reported that CLA supplementation does not enhance body fat loss during negative energy balance, particularly during caloric restriction (41,42).

Immune and inflammatory responses and CLA CLA has been reported to reduce inflammatory and improve immune responses, such as reducing colonic inflammation, decreasing antigen-induced cytokine production in immune-competent cells, and modulating the production of cytokines, prostaglandins, and leukotrien B_4, modulating TNF-α, cytokines (i.e., interleukin-1, 4, 6, or 8), prostaglandins, or nitric oxides, while reducing allergic type immune responses (20,43-52). However, others reported that CLA, particularly the *trans*-10,*cis*-12 isomers, induced inflammatory responses in white adipose tissue (53).

Bone health and CLA CLA has been reported to improve bone mass as reported by ash weights, bone density, bone mineral contents, bone dry weights, bone length, or calcium, magnesium, or phosphate contents. However, effects of CLA on body ash or bone mass have not been consistent (40,54-67). Recently, it was reported that CLA may interact with dietary calcium to improve bone mass (68). In fact, it has been reported that CLA influences calcium uptake in an isomer specific manner (69-71). Others reported that CLA reduced activities of osteoclasts to prevent bone resorption, although this finding has not been consistent when markers of bone resorption were tested by other groups (59,72,73).

CLA Isomer Interaction

With availability of the 2 major CLA isomers, a number of reports are now focused on the activities of these 2 isomers. While both major isomers have shown additive effects in most cancer models, either independent or antagonistic effects of these 2 isomers have also been reported (24-26). The *trans*-10,*cis*-12 isomer of CLA is identified as the active form for body compositional modulation, inhibition of hepatic stearoyl-CoA desaturase (SCD) activity and/or expression, and reduction of apolipoprotein B secretion (39,40,74). The *cis*-9,*trans*-11 isomer has been identified as active for growth promotion in rodents, improving lipoprotein profiles, and reducing TNF-α secretion which is more effective than the *trans*-10,*cis*-12 isomer (50,51,75). However, the 2 isomers appear to be antagonistic with regard to insulin resistance (76). Hence, the many physiological effects that are reported for CLA appear to be the result of multiple interactions of these 2 biologically-active CLA isomers (5,77).

Safety Issues

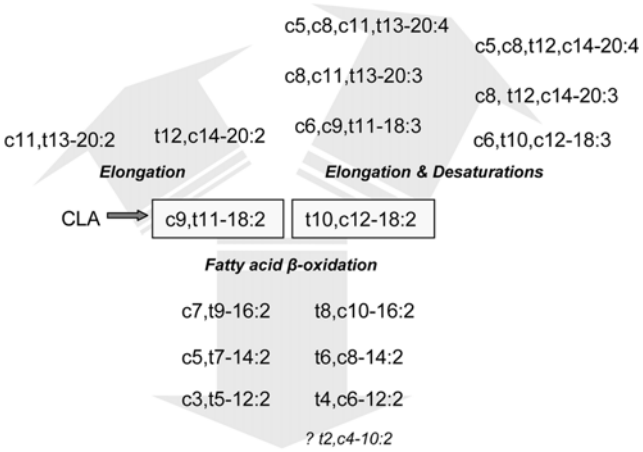
The main concerns over CLA use currently identified are lipodystrophy, fatty liver, and glucose intolerance (78-80). Lipodystrophy and fatty liver may be the results of CLA's pronounced effects of reducing body fat and increasing hepatic fatty acid synthesis in part mediated by peroxisome proliferator-activated receptors (PPARs) (79,81-83). These effects are more pronounced in mice than rats and other species including humans (84,85). Ten human clinical trials observed no changes in enzymatic markers for the liver and another study found no observable changes from the liver echograph (86), while one study observed increased enzymatic markers for the liver function and a single case report of human hepatitis has been reported (87,88). Based on animal studies, it is possible that CLA caused transient and reversible response in the liver (84,85, and unpublished observations).

Generally, it is suggested that a reduction of body fat will improve insulin sensitivity. However, supplementation of CLA has been linked to increased insulin resistance, although this finding was not consistent; as well as increased insulin resistance in normal animals but improved insulin resistance in an obese model (89-93). The exact mechanism by which CLA modulates glucose metabolism is not currently known. CLA may influence insulin resistance in part by enhanced fatty acid β-oxidation, by modulation of adipokines (leptin and adipokine) and cytokines (TNF-α), and/or by reduced the activities and expression of glucose transporter 4 from adipocytes (50,77,94-99). Similar to effects of CLA in the liver, this effect of CLA on insulin resistance has been suggested to be temporary and that CLA feeding may improve insulin resistance after a period of worsened insulin resistance (85,100). In addition, Poirier *et al.* (101) suggested that CLA increased insulin secretion due to pancreatic β-cell hyperplasia, although the significance of this study along with a duration link with CLA supplementation needs further evaluation.

Other Conjugated Fatty Acids

CLA's effect on body fat reduction has drawn significant attention for human application (5,20,102,103). However, compared to the body fat reduction seen in mice, CLA was less effective in humans (5,20,104,105). This may be due in part by discrepancies in doses administered, duration used, species differences, difference of dietary regimes as well as dietary compositions, and/or variation of fat metabolisms such as prevention of fat mass gain vs. elimination of fat accumulation (5,42,86,106,107). With this background, the effort to search for conjugated fatty acids with greater potency than CLA has been explored (39,108,109). Reported CLA metabolites are summarized in Scheme 1.

Elongated and/or desaturated metabolites It has been



Scheme 1. Metabolites of conjugated linoleic acid. Two main isomers of CLA, *cis*-9,*trans*-11 and *trans*-10,*cis*-12, in center, have been reported to be converted into a number of metabolites by elongation, desaturation, and/or fatty acid β-oxidation.

reported that both major CLA isomers can be converted into desaturated and elongated metabolites (110,111). Park *et al.* (108) also have reported elongated metabolites of CLA, conjugated eicosadienoic acid (CEA, conj. 20:2$\Delta^{c11,t13/\,t12,c14}$), from the liver homogenate. There were limited reports on bioactivites for these metabolites, only for inhibition of lipoprotein lipase from adipocytes and effects on Apolipoprotein B secretion from hepatocytes as summarized in Table 1 (39,108). The effects of CEA on lipoprotein lipase activity were comparable to CLA after 4-day treatment but not following 2-day treatment, although it was identified that CEA can be converted to CLA in the biological system (108). Thus it is apparent that CEA's effects were due to CLA itself. Conjugated eicosatrienoic acid (conj. 20:3$\Delta^{c8,t12,c14}$) only showed significant inhibition for lipoprotein lipase activity in 3T3-L1 adipocytes, although this was less active than CLA itself (108). Thus it is apparent that CLA's activity may be lost with additional metabolism, in particular during elongation and/or delta-6 desaturation. In addition both CEA and conjugated eicosatrienoic acid (conj. 20:3$\Delta^{c8,t12,c14}$) did not influence apolipoprotein B secretion in human hepatocytes while CLA significantly reduced it (39).

Other CLA metabolites CLA is reported to undergo through fatty acid β-oxidation and generate shorter chain

Table 1. Summary of activities of conjugated fatty acids

Fatty acids		Conc. (μM)	LPL activity[1)]	Apo B secretion[2)]
Conjugated	Non-conjugated			
18-Carbon fatty acids			(% control)	
CLA,18:2$\Delta^{c9,t11/t10,c12}$		100	17-34[3)]	78[3)]
t10,c12-CLA, 18:2$\Delta^{t10,c12}$		50	17-45[3)]	46-81[3)]
c9,t11-CLA,18:2$\Delta^{c9,t11}$		50	99	91
	Linoleic acid, 18:2$\Delta^{c9,c12}$	50	92	119
20-Carbon fatty acids				
CEA, 20:2$\Delta^{c11,t13/\,t12,c14}$		100	46[3)]	99
		200	26[3)]	ND[4)]
c11,t13-CEA, 20:2$\Delta^{c11,t13}$		50	82	103
		100	88	ND
	EA, 20:2$\Delta^{c11,c14}$	50		136
		100	107	ND
		200	84	ND
CETA, 20:3$\Delta^{c8,t12,c14}$		50	104	127
	ETA, 20:3$\Delta^{c8,c11,c14}$	50	123	145
19-Carbon fatty acids				
CNA, 19:2$\Delta^{c10,t12/\,t11,c13}$		50	53[3)]	77[3)]
		100	18[3)]	69[3)]
	NA, 19:2$\Delta^{c10,c13}$	50	114	101
		100	110	98
21-Carbon fatty acids				
CHDA, 21:2$\Delta^{c12,t14/\,t13,c15}$		50	125	ND
		100	108	ND
	HDA, 21:2$\Delta^{c12,,c15}$	50	105	ND
		100	144	ND

[1)]Data are from (40, 65, 108, 109, 115).
[2)]Data are from (39).
[3)]Significantly different from representative controls at $p<0.05$.
[4)]Not determined.

conjugated fatty acids (Scheme 1 bottom arrow). Conjugated hexadecadienoic (conj. Δ16:2), tetradecadienoic (conj. Δ14:2), and dodecadienoic (conj. Δ12:2$^{c3,t5/t4,c6}$) acids were reported as metabolites of CLA (108, 110, 112-114). Park *et al.* (108) observed accumulation of conjugated dodecadienoic acids (conj. Δ12:2$^{c3,t5/t4,c6}$), and suggested that further fatty acid β-oxidation of these 2 conjugated fatty acids may not be efficient with enoly-CoA isomerase due to the presence of conjugated double bonds. In addition, since the amount of *cis*-3,*trans*-5 conjugated dodecadienoic acid was greater than *trans*-4,*cis*-6 conjugated dodecadienoic acid, it is possible that the *trans*-4,*cis*-6 conjugated dodecadienoic acid may be oxidized further to conjugated diene 10:2 (conjugated decadienoic acid), although it has not been confirmed (108). Biological significances of these CLA metabolites have not yet been tested.

Odd-carbon conjugated fatty acids Two odd-carbon conjugated fatty acids, conjugated heneicosadienoic acid (conj. 21:2Δ$^{c12,t14/\ t13,c15}$) and conjugated nonadecadienoic acid (CNA, conj. 19:2Δ$^{c10,t12/\ t11,c13}$) were also tested for their bioactivities (108,109). Conjugated heneicosadienoic acid had no effects on lipoprotein lipase activity in adipocytes (108). However, CNA showed greater inhibitory activity of lipoprotein lipase compared to CLA, while the effects of CNA on hepatic apolipoprotein B secretion were similar to those of CLA (Table 1) (39,109,115). Effects of CNA and CLA have been further compared with regard to the inhibition of stearoly-CoA desaturase (SCD). This is the key enzyme in the biosynthesis of monounsaturated fatty acids and subsequently these monounsaturated fatty acids are incorporated into membrane phospholipids, triglycerides, and cholesterol esters (116,117). As shown in Fig. 1, both CLA and CNA significantly inhibited activities of SCD, where the active CLA isomer for this activity has been suggested to be the *trans*-10,*cis*-12 isomer (74). Since the CNA used here was a mixture of 2 main isomers (*cis*-10,*trans*-12 and *trans*-11,*cis*-13), 100 μM CNA represented equivalent doses for 50 μM *trans*-10,*cis*-12 CLA isomer and at this concentration CNA significantly reduced activity of SCD, even when compared to CLA.

Even though Kang *et al.* (118) previously reported the independent effects of CLA from SCD, it is expected that CNA would be more effective in reducing body fat in animal model based on its greater inhibitory activities on lipoprotein lipase and SCD compared to CLA. In fact, in the mouse model, CNA reduced total body fat and adipose tissue masses compared to CLA (Fig. 2, Exp. 1) and non-conjugated control nonadecadienoic acid (Fig. 2, Exp. 2) (109,119). This suggests that there is potential use of CNA, although further investigations into the mechanism of CNA in comparison to CLA as well as identification of active CNA isomers are needed in the near future to support the applications of these unusual fatty acids.

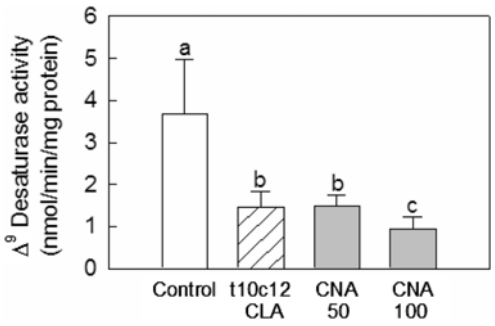


Fig. 1. Effects of conjugated nonadecadienoic acid (CNA) on stearoyl-CoA desaturase. Mouse liver microsomal fractions were treated with fatty acid-albumin complexes before determining the activities of stearoyl-CoA desaturase. Means with different letters are significantly different at $p<0.05$ ($n=4$). Numbers indicate concentrations used as μM. *Trans*-10,*cis*-12 CLA was 50 μM.

Conclusion

CLA is an unusual fatty acid with a wide range of biologically beneficial effects. With potentially wide applications as supplements or in food, we still need to understand how CLA exerts its multiple biological activities. Moreover, possible pharmacological applications are based on comparison between CLA and other conjugated fatty acids. Further studies are necessary for effective as well as safe applications of CLA to improve human health.

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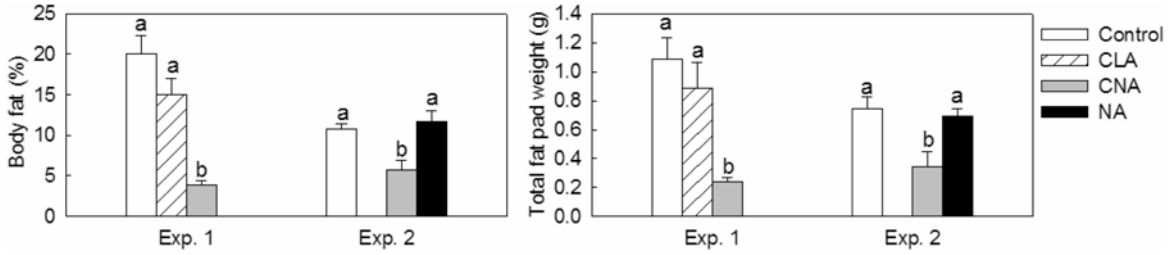

Fig. 2. Effects of conjugated nonadecadienoic acid (CNA) on body fat reduction (A) and adipose tissue weights (B). Experiment 1 used female ICR mice fed 0.3% CNA or CLA for 14 days ($n=5$-6). Experiment 2 used male ICR mice fed 0.2% CNA or non-conjugated *cis*-10,*cis*-3 nonadecadienoic acid (NA, 19-carbon non-conjugated control) for 10 days ($n=7$-8). Body fat data in Exp.1 is taken from Park and Pariza (109). Mean±SE. Bars with different letters in each experiments are significantly different at $p<0.05$. CLA, conjugated linoleic acid; CNA, conjugated nonadecadienoic acid; NA, non-conjugated *cis*-10,*cis*-3 nonadecadienoic acid.

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