MINIREVIEW

Mechanisms of Action of Conjugated Linoleic Acid: Evidence and Speculation

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MICHAEL W. PARIZA,*,[1] YEONHWA PARK,* AND MARK E. COOK*,†

Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, Madison, Wisconsin 53706; and †Department of Animal Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Abstract. ***Conjugated linoleic acid*** **(CLA) has been shown to inhibit carcinogenesis and atherosclerosis, enhance immunologic function while protecting against the catabolic effects of immune stimulation, affect body composition change (reducing body fat gain while enhancing lean body mass gain), and stimulate the growth of young rats. We discuss possible biochemical mechanisms that underlie these physiological effects. We emphasize the importance of considering the effects, both individually and combined, of the two CLA isomers (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) that have been shown to exhibit biological activity and which appear to exert their effects via different biochemical mechanisms.** [P.S.E.B.M. 2000, Vol 223]

The term conjugated linoleic acid refers to a class of positional and geometric conjugated dieonic isomers of linoleic acid (Fig. 1). We coined the acronym CLA when we reported that conjugated linoleic acid, isolated from grilled beef or produced by base-catalyzed isomerization of linoleic acid, was an effective inhibitor of benzo[a]pyrene-initiated mouse epidermal neoplasia (1). This was followed by our observation that feeding CLA (0.5% of diet) to rodents or chickens protected them from the catabolic effects of immune stimulation (2, 3). CLA enhanced feed efficiency in young rats (4) indicating that it may regulate energy metabolism and nutrient partitioning, a conclusion that has been verified (5). We also found that dietary CLA reduced blood lipids and the development of atherosclerosis in rabbits fed an atherogenic diet (6). These findings have been confirmed independently and expanded in different models by other investigators (7) (for an updated listing of the scientific literature on CLA since 1987, which expands weekly, see the internet address http://www.wisc.edu/fri/clarefs.htm).

Taken together, findings to date indicate a broad range of biological activities for CLA that beg the question of underlying biochemical mechanism. How is it possible for CLA to induce so many seemingly unrelated physiological effects? There are several factors to consider.

CLA Is Not a Single Substance

The term conjugated linoleic acid and its acronym CLA refer generally to mixtures of positional and geometric conjugated dieonic isomers of linoleic acid. Many CLA isomers are produced when linoleic acid is heated in the presence of base (8, 9). Numerous CLA isomers are also found in milkfat, cheese, and beef (10). However, the *cis*-9,*trans*-11 isomer is the principal dietary form of CLA (9–13).

The *cis*-9,*trans*-11 isomer of CLA is produced in the rumen of cattle and other ruminant animals during the mi-

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[1] To whom requests for reprints should be addressed at Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, 1925 Willow Drive, Madison, WI 53706. E-mail: mwpariza@facstaff.wisc.edu

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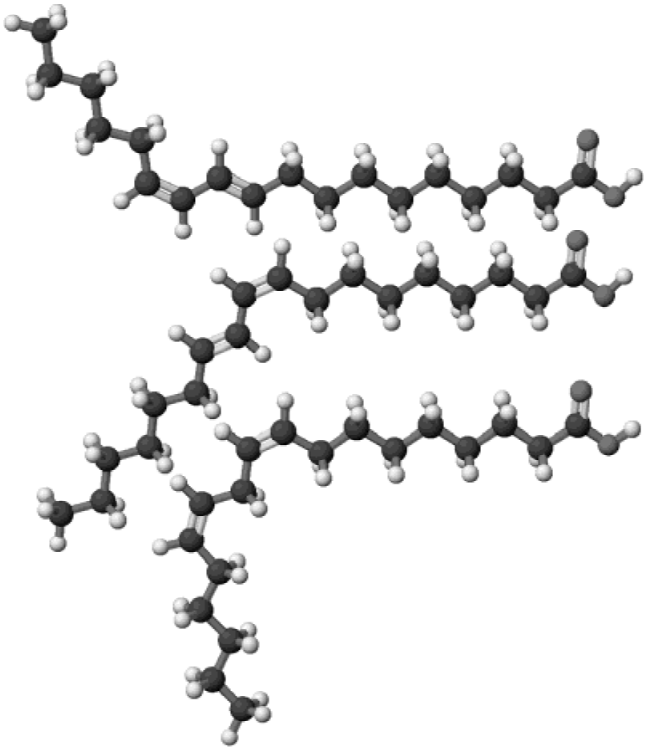

Figure 1. Structures of (top) *trans*-10,*cis*-12 CLA, (middle) *cis*-9,*trans*-11 CLA, and (bottom) linoleic acid. (From (58), reprinted with permission.)

crobial biohydrogenation of linoleic and linolenic acids (14). Thereafter *cis*-9,*trans*-11 CLA may be absorbed directly (4) or biohydrogenated to vaccenic acid (*trans*-11-octadecenoic acid). Vaccenic acid, after absorption, may then be converted by δ-9 desaturase within mammalian cells back to *cis*-9,*trans*-11 CLA (15–17).

In addition to vaccenic acid, *trans*-10-octadecenoic acid is also found in cow's milk (18). Verhulst *et al*. (19) isolated a microorganism that converts linoleic acid to *trans*-10,*cis*-12 CLA, so it is likely, by analogy to vaccenic acid, that *trans*-10-octadecenoic acid may form in the rumen *via* microbial metabolism of linoleic acid to *trans*-10,*cis*-12 CLA, which is then biohydrogenated at the *cis*-12 bond. Since mammals do not possess δ-12 desaturase, it follows that the *trans*-10,*cis*-12 CLA reported in ruminant tissues [and which apparently can be increased by dietary manipulation (Dhiman T, Griinari M, personal communication)] would originate from *trans*-10,*cis*-12 CLA that was absorbed from the gastrointestinal tract. However, Park and Pariza (20) presented evidence indicating that commercial horse sera can sometimes contain substantial levels of apparent *trans*-10,*cis*-12 CLA. Since the horse has a hindgut fermentation area (rather than a rumen) where long-chain fatty acid absorption is minimal, the finding of apparent *trans*-10,*cis*-12 CLA in a sample of horse sera indicates that the origin of CLA isomers in the blood may be more complex than currently thought.

Uchida (21) reported the apparent *de novo* synthesis of CLA isomers by *Pediococcus homari* (as opposed to the generation of CLA isomers by isomerizing linoleic or linolenic acids, as is the case with all other known CLA-producing microorganisms). The origins of other CLA isomers that have been reported to occur naturally in milkfat (10) are not known, but one may conjecture that these also result largely, if not completely, from bacterial metabolism in the rumen.

The many physiological effects of CLA in animal models reported to date have been produced by feeding mixtures of CLA isomers that contain mostly *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA in approximately equal amounts, with other CLA isomers at considerably lower levels (10, 11). For example, CLA that we typically produce for experimental use consists of the *cis*-9,*trans*-11 (40.85–41.1%), *trans*-10,*cis*-12 (43.5–44.9%), and *trans*-9,*trans*-11/*trans*-10,*trans*-12 (4.6%–10%) isomers (5, 9, 11). One should be cautioned, however, that some commercial CLA preparations contain additional isomers with conjugated double bonds at the 8, 10, or 11, 13 positions (11). Obviously the presence of additional CLA isomers will complicate data interpretation.

Physiological Effects of CLA Isomers

Reports are now indicating that the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers induce different effects. Park *et al*. (22) provided evidence indicating that the CLA-associated body composition changes in mice result from feeding the *trans*-10,*cis*-12 CLA isomer. Table I is taken from that re-

Table I. Evidence that the t10,c12 CLA Isomer Effects Body Composition Change in Mice[a]

	ECW (g)[a]	% fat	% water	% protein	% ash
Control	27.43* ± 1.21	22.27* ± 1.80	54.30* ± 1.35	16.26* ± 0.49	3.29* ± 0.13
CLA-1[b]	24.28† ± 0.76	6.69‡ ± 0.86	65.59‡ ± 0.68	19.04† ± 0.24	3.78† ± 0.10
CLA-2[c]	25.53*† ± 0.59	13.08† ± 1.66	60.99† ± 1.14	18.09† ± 0.50	3.54*† ± 0.13
CLA-3[d]	23.44† ± 0.92	6.80‡ ± 1.26	65.35‡ ± 1.13	19.33† ± 0.29	3.83† ± 0.08

[a] From (22). Female ICR mice were fed control diet or diet supplemented with 0.5% CLA-1, 0.3% CLA-2, or 0.25% CLA-3, for 4 weeks. Reported body composition values are means ± SE (n = 5–6). In each column, means with different symbols are significantly different (P < 0.05). See Ref. 22 for further experimental detail.
[b] CLA-1 preparation consisted of 41.1% c9,t11 plus 43.5% t10,c12.
[c] CLA-2 preparation consisted of 72.4% c9,t11 plus 13.0% t10,c12.
[d] CLA-3 preparation consisted of 16.2% c9,t11 plus 79.2% t10,c12.
[e] ECW, empty carcass weight.

port. CLA preparations were enriched for the *cis*-9,*trans*-11 CLA isomer, or the *trans*-10,*cis*-12 CLA isomer. Body composition changes, exhibited as reduced body fat, enhanced body water, enhanced body protein, and enhanced body ash, were associated with feeding the *trans*-10,*cis*-12 CLA isomer. Similar findings have been reported for hamsters (23).

Park *et al*. (22) also conducted experiments with cultured 3T3-L1 mouse adipocytes. In these cells the *trans*-10,*cis*-12 isomer reduced lipoprotein lipase activity as well as the concentrations of intracellular triacylglycerol and glycerol. By contrast the *cis*-9,*trans*-11 and *trans*-9,*trans*-11 CLA isomers did not affect those biochemical activities. Similar findings have been reported by Lin *et al*. (24).

Lee *et al*. (25) provided limited evidence indicating that the *trans*-10,*cis*-12 CLA isomer decreased the expression of hepatic stearoyl-CoA desaturase mRNA in mice, whereas enzymatically synthesized *cis*-9,*trans*-11 CLA was not active in this regard. In collaborative studies with Professor James Ntambi of the University of Wisconsin-Madison Department of Biochemistry, we are finding that highly purified *trans*-10,*cis*-12 CLA, but not *cis*-9,*trans*-11 CLA, decreases the expression of stearoyl-CoA desaturase in cultured 3T3-L1 adipocytes (unpublished data). This may relate to the report (17) that the *trans*-10,*cis*-12 CLA isomer depressed milk fat synthesis in cows.

Yotsumoto *et al*. (26) reported that the *trans*-10,*cis*-12 CLA isomer reduced apolipoprotein B secretion in cultured human hepatoma HepG2 cells, thereby confirming and expanding upon the findings of Lee (27) and Pariza and Lee (28) that a mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers reduced apolipoprotein B secretion in this cell line. *Trans*-10,*cis*-12 CLA reduced triacylglyceride secretion in HepG2 cells (24, 26) although the effect of this isomer on triacylglyceride synthesis in this cell line was less clear (compare findings in references 24 and 26). It is not yet known which CLA isomer(s) are involved in other reported effects on atherosclerosis in various animal models (6, 29–31).

DeVoney *et al*. (32) provided evidence indicating that the *trans*-10,*cis*-12 CLA isomer alters lymphocyte blastogenesis. Further research on the effects of this isomer on the immune system is in order. In this regard mixtures of CLA isomers (mostly *cis*-9,*trans*-11 and *trans*-10,*cis*-12) have been shown to enhance the immune system (2, 33–35), reduce the catabolic effects of immune stimulation (2, 3), and reduce the release of prostaglandin E2 and leukotriene B4 from antigen-challenged lung, trachea, and bladder in the guinea pig (36).

In summary, the reported effects of CLA on lipid metabolism and body composition, and at least some of the effects of CLA on the immune system, appear to be due to *trans*-10,*cis*-12 CLA. What, then, might the *cis*-9,*trans*-11 CLA isomer do?

Ip has obtained evidence indicating that the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers may be equally effective in inhibiting carcinogenesis (Ip C, personal communication). We have obtained limited evidence indicating that *cis*-9,*trans*-11 CLA may be important in effecting the CLA-induced growth enhancement in young rodents (4) and further that *trans*-10,*cis*-12 CLA may interfere with the growth enhancement induced by the *cis*-9,*trans*-11 isomer. Belury and Vanden Heuvel (37) found that *cis*-9,*trans*-11 CLA is a potent activator and high-affinity ligand for peroxisome proliferator–activated receptor-α (PPAR-α), but a CLA isomer mixture containing both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA did not induce peroxisome proliferation in rat liver (38). Whether other CLA isomers also induce physiological effects is not yet known.

As a result of these findings, the apparent multifunctionality of CLA has become more intriguing and less perplexing. Different isomers appear to produce different effects. It is also virtually certain that more than one biochemical mechanism is involved in the various physiological effects of CLA. In fact, more than one biochemical mechanism may even be involved in the effects of individual CLA isomers, in particular the *trans*-10,*cis*-12 isomer.

Multiple Biochemical Mechanisms

Our working hypothesis is that the numerous biological effects reported for CLA cannot be explained by a single biochemical mechanism. A major direct line of support for this conclusion is that the *trans*-10,*cis*-12 and *cis*-9,*trans*-11 isomers appear to produce different effects, and it is difficult to imagine a single mechanism that accounts for this observation. By extension, even those effects that are clearly attributed to a single isomer may not all be caused by the same biochemical mechanism. For example, we have obtained evidence indicating that *trans*-10,*cis*-12 CLA directly inhibits the activity of stearoyl-CoA desaturase. Again, it is difficult to imagine how this finding alone could explain the range of physiological effects of this CLA isomer.

One might speculate that the inhibition of carcinogenesis by CLA could result from the combined effects of a number of CLA activities, possibly including direct effects of one or more CLA isomers/metabolites on cell differentiation (39), effects of CLA on vitamin A metabolism that would also influence cell differentiation (40), and effects of one or more CLA isomers on prostaglandin metabolism (41, 42), which may also influence cancer development at some sites (43). Similarly, the effects of CLA on lipid metabolism may be mediated by effects of the *trans*-10,*cis*-12 isomer and/or its metabolites on both the regulation and biochemical activities of key adipocyte and skeletal muscle enzymes (5, 22, 25) as well as effects on adipocyte differentiation (37, 44, 45).

Accordingly, we should now consider how CLA might effect the complex signaling required for such putative multiple biochemical mechanisms.

CLA and Eicosanoids

Given the structural similarities between the CLA isomers and linoleic acid, it seems likely that at least some of the activities of CLA may be mediated *via* modification of intracellular signaling by eicosanoids and other lipid mediators. In this regard CLA could effect the synthesis and/or action of these mediators. Alternatively, the CLA isomers could be desaturated and elongated, then further metabolized to produce various CLA-derived eicosanoids and other novel mediators that would exhibit biological activities in their own right. Evidence exists for both of these propositions. (The term eicosanoid refers to metabolites of arachidonic acid, and since 20 carbon derivatives of CLA are also isomers of arachidonic acid, we believe that the term CLA-derived eicosanoid is justified. The possibility that CLA metabolites of different chain lengths may also be biologically active should be considered as well).

Research from a number of laboratories indicates that CLA effects the synthesis of prostaglandins, in particular PGE_2 (46). Sebedio *et al*. (47) have provided evidence indicating the both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA are elongated and desaturated in manners analogous to that of linoleic acid, hence providing precursors for putative CLA-derived eicosanoids. Pariza *et al*. (48) provided evidence indicating that conjugated eicosadienoic acid, CEA, produced by heating eicosadienoic acid in the presence of base, produces changes in body composition in mice that are similar to that of CLA. Hence it seems likely that the mechanisms of action of the CLA isomers involve, at least in part, effects both on eicosanoid signaling, as well as possibly unique signaling by CLA-derived eicosanoids. Altered eicosanoid signaling, and CLA-derived eicosanoid signaling, could in turn effect a range of biological activities including lipid metabolism and cytokine synthesis/function (49).

It should also be noted that *trans*-10,*cis*-12 CLA appears to be metabolized more rapidly than *cis*-9,*trans*-11 CLA, particularly in skeletal muscle (45). Whether this is due to enhanced elongation/desaturation, enhanced β-oxidation, or both, is not yet known. However, given obvious structural differences (Fig. 1), one might expect CLA-derived eicosanoids that originate from *trans*-10,*cis*-12 CLA to be functionally distinct from those that might arise from *cis*-9,*trans*-11 CLA.

CLA and Cytokines

Cytokines are hormone-like mediators of immunity and inflammation that are produced by macrophages and other immune cells when they are stimulated. Tumor necrosis factor-α (TNF-α), along with interleukin-1 (IL-1), induce a number of effects in immune cells including the inflammatory response (50). However, these cytokines also produce biochemical changes in other cells, for example the induction of catabolism in skeletal muscle (51) and changes in cell surface proteins (52, 53).

Interestingly, virtually every cell in the body has receptors for TNF-α, and many types of cells (e.g., nerve cells, adipocytes) can also produce this cytokine (51). It is also noteworthy that both the synthesis and action of TNF-α and IL-1 are regulated by eicosanoids, in particular prostaglandin E_2 (PGE_2) (50).

TNF-α appears to be a key mediator in many chronic pathologies including cachexia (54), atherosclerosis (55), carcinogenesis (56, 57), and (paradoxically) obesity (51). The association of TNF-α with so many biological and physiological processes has led Hotamisligil and Spiegelman (51) to conclude that this cytokine produces a ". . . bewildering array of biochemical changes in a wide variety of cells . . . attributable to its capacity for using multiple signaling pathways through its cell surface receptors." Hence TNF-α, like CLA, is multifunctional.

The data of Figure 2 (from Ref. 42) are germane to this discussion. Mice were fed control diet or diet supplemented with 0.5% CLA for 32 days, then injected with TNF-α as indicated. The CLA-fed mice experienced less weight loss indicating that they were partially protected against the cachexia that was induced by the cytokine. These results provide evidence indicating that CLA may modulate a cellular response to TNF-α, possibly through the regulation of eicosanoid/CLA-derived eicosanoid production and/or type.

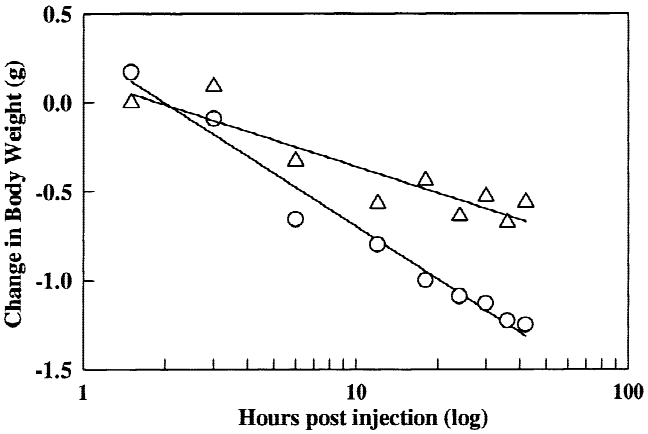

Figure 2. Dietary CLA protects against cachexia induced by TNF-α. Forty 4-week-old male ICR mice (Harlan Sprague-Dawley, Madison, WI) were housed, two per cage, in a windowless room on a 12:12-hr light:dark cycle, in strict accord with guidelines established by the Research Animal Resources Center of the University of Wisconsin-Madison. The animals were divided into two groups of equal size. One group was fed a semipurified diet (TD94060, Harlan Teklad, Madison, WI). The other group was fed the same diet supplemented with 0.5% CLA, for 32 days (fresh diet was provided each day). At Day 28, half of the animals in each group were injected (i.p.) with recombinant murine TNF-α (200 µg/kg body weight) (R&D Systems, Minneapolis, MN) in phosphate-buffered saline (PBS); the remaining mice were injected with PBS alone. Points represent the differences between TNF-α–treated mice and their respective PBS control counterpart for 42 hr postinjection. The r^2 values are 0.9742 for control (○) and 0.8320 for CLA (δ). Using regression analysis with indicator variables, tests were conducted for the null hypotheses that each slope equaled 0 and for the null hypothesis that the two slopes were the same. The slopes are significantly different from each other and from 0 ($P < 0.05$). (From (42).)

Our working hypothesis is that some of the multifunctionality of CLA may be explained by the effects of the CLA isomers on the cellular responses to TNF-α, possibly through altered eicosanoid signaling, CLA-derived eicosanoid signaling, or both (2, 3, 5, 22, 42, 49). This proposition is perhaps most defensible with regard to the effects of *trans*-10,*cis*-12 CLA, but possible effects of *cis*-9,*trans*-11 CLA and other isomers on signaling mediated by TNF-α and possibly other cytokines should not be discounted.

Our expectation is that evidence for this hypothesis will continue to mount and will be a major theme when this review is updated three years hence.

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