Perspective on the safety and effectiveness of conjugated linoleic acid\textsuperscript{1–4}

Michael W Pariza

ABSTRACT
The amount of scientific literature on conjugated linoleic acid (CLA) is growing at a phenomenal rate. Animal studies and clinical trials indicate the possibility that CLA could be useful in improving human health in a number of areas, eg, controlling body fat gain and enhancing immunity while also reducing inflammation and other adverse effects typically associated with immune enhancement. The background of this growing research field and mechanistic insights from animal and cell culture experiments are briefly reviewed. Experimental and clinical data relating to the safety and effectiveness of CLA in humans are presented and discussed. Am J Clin Nutr 2004;79(suppl):1132S–6S.

KEY WORDS Conjugated linoleic acid, immunity, body-weight control, diabetes, atherosclerosis

INTRODUCTION
In 1978, Pariza et al (1) reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis. The finding of mutagens in grilled beef was confirmatory, but evidence of a mutagenesis inhibitor was a novel discovery that had not been previously reported. That study (1) concluded with a speculative prediction, “...it may also be found that the mutagenic inhibitory activity inhibits carcinogenesis.” Subsequently, Pariza et al (2) established that this speculation was indeed the case and went on to identify the new anticarcinogen as conjugated linoleic acid (CLA) (3).

The discovery that CLA inhibited carcinogenesis in several animal models led to an investigation into the biochemical mechanisms of action of CLA. In the course of my work and the work of others, a number of additional potential applications were identified, as indicated in Table 1 (4–17).

The potential use of CLA to control body fat gain in humans and animals has received the most recent attention in both popular and scientific publications. However, the application of CLA to other areas could be of equal or greater health importance, eg, enhancing immunity while also reducing inflammation and other adverse effects typically associated with immune enhancement (11–17).

TWO BIOLOGICALLY ACTIVE CONJUGATED LINOLEIC ACID ISOMERS

The numerous CLA effects listed in Table 1 beg the question of how they all could be induced by a single substance. Mechanistic considerations complicate matters further, in that there is no known common biochemical pathway on which CLA could act to mediate these many physiologic effects. So how does CLA do it?

Much of the answer lies in the fact that CLA is not a single substance. CLA is a collective term for a class of conjugated dienic isomers of linoleic acid. It is possible that a number of these CLA isomers have biological activity. However, all of the known physiologic effects of CLA are induced by 2 isomers: \(c_{9,11}\)-CLA and \(c_{10,12}\)-CLA (Figure 1) (4).

In some cases an effect is produced by one of these isomers acting alone. For example, it is apparent that \(c_{10,12}\)-CLA is solely responsible for the reduction of body fat gain (18), whereas the \(c_{9,11}\)-isomer enhances growth and feed efficiency in young rodents (4). In other cases the isomers act together to induce an effect. For example, both \(c_{9,11}\) and \(c_{10,12}\)-CLA appear to be equally effective in inhibiting chemically induced mammary carcinogenesis in rodent models (19), in part by inhibiting angiogenesis (20), whereas \(c_{10,12}\)-CLA appears to be more effective than \(c_{9,11}\)-CLA in inhibiting the proliferation of MCF-7 breast cancer cells by way of elicitation of a p53 response (21). In still other instances the 2 biologically active CLA isomers appear to act in apparent opposition (22, 23). Hence, the multiple physiologic effects that are reported for CLA (Table 1) appear to be the result of multiple interactions of the biologically active CLA isomers with numerous metabolic signaling pathways (4).

BODY FAT REDUCTION BY CONJUGATED LINOLEIC ACID

Figure 2 depicts a model for the proposed mechanism of body fat control by \(c_{10,12}\)-CLA, the isomer responsible for inducing this physiologic effect (18). At this time the clearest indication is that \(c_{10,12}\)-CLA inhibits adipocyte lipoprotein lipase activity, thereby reducing lipid uptake into adipocytes (18, 24).\(c_{10,12}\)-CLA also affects preadipocyte differentiation

1 From the Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin–Madison.
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4 Reprints not available. Address correspondence to MW Pariza, Food Research Institute, University of Wisconsin–Madison, 1925 Willow Drive, Madison, WI 53706. E-mail: mwpariza@wisc.edu.
whereas there is only limited experimental support for a possible effect of CLA on lipolysis in adipocytes (26). CLA also appears to enhance fat oxidation in skeletal muscle (24), which is likely to be mechanistically related to the enhancement of oxygen consumption and energy expenditure in Otsuka Long Evans Tokushima Fatty rats fed CLA-supplemented diets (27).

The data underlying the biochemical mechanisms summarized in Figure 2 come mainly from studies with mice and cultured mouse 3T3-L1 adipocytes, but it should be noted that t10,c12-CLA also reduced triacylglycerol accumulation in cultured human adipocytes and in markers of differentiation, as well as the uptake and oxidation of glucose and oleate in cultured human preadipocytes (28, 29).

The findings to date provide insight for the design of human clinical trials. First, it is important to emphasize that in many animal models dietary CLA induces substantial reductions in body fat without substantially reducing body weight. For example, male or female weanling ICR mice fed diet supplemented with 0.5% CLA (composed approximately of equal mixture of c9,t11- and t10,c12-isomers) for 4 wk exhibited, respectively, 57% or 60% reductions in body fat relative to controls, whereas there were no significant differences in body weights relative to controls (24).

Second, in most animal models the reduction in body fat appears to be due mostly to reductions in body fat accretion, not reductions in body fat that had already accumulated before the initiation of the experiment. In fact mice are the only species that have clearly been shown to lose accumulated body fat when fed diet supplemented with t10,c12-CLA (4). Researchers speculated that this result could occur because mice may depend more than larger species on fat combustion for energy and, hence, are more sensitive to CLA-induced reductions in fat storage in adipocytes. Finally, there are considerable species differences with regard to reductions in body fat accretion in response to dietary CLA (30). Mice are the most sensitive, followed in order by hamsters and rats. Direct comparisons with pigs versus rodents have not been performed, but it would appear that pigs are likely to be intermediate, ie, more like hamsters than mice or rats in this regard (31).

Most of the published human clinical trials to date were designed to test the hypothesis that CLA ingestion will reduce accumulated body fat in adult humans (32). In general the protocols involved giving encapsulated CLA as a free fatty acid at an amount of about 3 g/d, with the highest intake in one study being 7.2 g/d (32). The results of those trials are mixed, but in general when the highest-quality commercially available grades of CLA were used, it was indicated there was significant body fat mass reduction relative to placebo controls (32). It is also indicated that

<table>
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<tr>
<th>TABLE 1</th>
<th>Some of the reported physiologic effects of conjugated linoleic acid$^1$</th>
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<td>Provides anticarcinogenic effect</td>
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<td>Enhances immune function</td>
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<td>Reduces inflammation</td>
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<td>Reduces asthma in animal models</td>
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<td>Reduces atherosclerosis</td>
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<td>Enhances growth of young rodents</td>
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<td>Reduces body fat gain</td>
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<td>Enhances lean body mass gain</td>
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<td>Reduces negative effects of weight-loss diets</td>
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<td>Reduces symptoms of diabetes in some models</td>
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$^1$From references 4–17.
CLA could prove to be most effective in reducing fat mass and increasing lean mass when combined with enhanced physical activity. However, CLA intake was not associated with significant reductions in body weight, which is also consistent with animal studies (4, 24).

Kamphuis et al (33, 34) approached the issue differently, designing a protocol to test whether CLA could reduce the regain of body fat or body weight in overweight adults who were first subjected to a very-low-calorie weight loss diet. Subjects were given the very-low-calorie diet for 3 wk followed by a 13-wk intervention period during which subjects ate ad libitum but were given CLA (1.8 or 3.6 g) or placebo each day. Subjects taking CLA (either dose level) exhibited significantly greater regain of fat-free mass relative to control subjects, and the increased fat-free mass in the subjects ingesting CLA was reflected in significantly enhanced resting metabolic rate. Interestingly, measures of appetite (hunger, satiety, and fullness) were also favorably, dose-independently affected by CLA ingestion. However, as one might predict, the regain of body weight per se was not reduced by CLA ingestion. Blood lipids, glucose, and insulin were not affected by CLA ingestion.

The findings of Kamphuis et al (33, 34) support the conclusion that CLA might be most effective in controlling body fat accretion in anabolic humans, rather than reducing accumulated body fat per se.

**CONJUGATED LINOLEIC ACID SAFETY**

Safety is the paramount consideration. A substance that is not safe when used as intended should not be sold as a food ingredient or dietary supplement, irrespective of whether it is physiologically effective. Animal tests are typically used in preclinical evaluations of the safety of new food ingredients. CLA safety has been evaluated in several well-conducted animal toxicologic studies. Scimeca (35) conducted a 36-wk feeding trial in which Fischer 344 rats were fed either control diet or diet supplemented with 1.5% CLA, a level 30 times greater than humans would ingest at 3 g CLA/d. Food disappearance, body weights, caged side examinations, and hematologic and histopathologic analyses of 15 major organs were conducted. No adverse effects were observed.

O’Hagan and Menzel (36) conducted a subchronic 90-d oral rat toxicity study, accompanied by a battery of in vitro genotoxicity studies that are typical for assessment of food ingredient safety, on a commercial preparation of CLA that consisted of equal amounts of the 11- and 12-CLA isomers in the form of glycerides (rather than free fatty acids). They concluded that the no observed adverse effect levels for male and female rats were 2433 and 2728 mg·kg body wt·d\(^{-1}\), respectively.

In addition to these peer-reviewed published studies, there are 2 abstracts of note that relate to CLA safety assessment in animal models. Schulte et al (37) and Pfeiffer et al (38) conducted comprehensive toxicologic evaluations of CLA methyl esters in dogs and pigs, using standard toxicologic protocols approved by European Organisation for Economic Co-operation and Development Guidelines. They concluded that CLA methyl esters did not produce adverse effects in these species even when fed at 5% of the diet. These findings should, of course, be considered preliminary until the full-length manuscripts are available for review.

A number of human clinical trials that relate to safety and efficacy were also conducted. In designing human trials, CLA quality is a topmost issue. The most successful clinical studies were conducted with high-quality CLA preparations that consist almost entirely (ie, >90%) of the 2 biologically active isomers (Figure 1) in approximately equal amounts (ie, about 45% each), as reviewed by Gaulier et al (32). It should also be noted that such high-quality CLA, when consumed at 3–6 g/d, does not appear to induce adverse effects in humans (7, 11, 33, 34).

Despite these conclusions some researchers have recently raised concerns about the potential safety of CLA for humans (39–41). The concerns include the induction of fatty liver, insulin resistance, and lipodystrophy in mice fed CLA-supplemented diets and in some human trials enhanced C-reactive protein, lipid peroxidation, unfavorable changes in serum lipids, and reduced milk fat. I consider these concerns in order, in light of the overall scientific literature database on CLA.

Fatty liver is induced in mice fed CLA-supplemented diets [reviewed in Pariza et al (4)]. However, this finding appears limited to mice in that it has not been reported for other species. Hamsters fed CLA and female rats fed diet supplemented with 15% CLA also exhibit enlarged livers, but this result is due to hypertrophy, not fat accumulation (36). It should be noted that neither fatty liver nor liver hypertrophy is considered by toxicologists to be a toxic effect (42). O’Hagan and Menzel (36) reported that the liver hypertrophy observed in female rats fed diet supplemented with 15% CLA was completely reversible when the animals were switched to a diet free of CLA.

The induction of mild insulin-resistance in mice was studied extensively and appears to be related to experimental conditions. For example, the effect is observed in mice fed diet supplemented with 1% CLA for 39 d (43) but not in mice fed diet supplemented with 0.5% CLA for 4 or 49 d (26) (0.5% CLA is sufficient to maximally induce body fat reduction in this species). Additionally, diabetic C57BLKS-Lepr\(^{db}/lepr^{db}\) mice fed a diet supplemented with 1.2% CLA for 23 wk exhibited significant reductions in blood glucose and improved insulin sensitivity even in the face of an oral glucose challenge (44, 45). One could speculate that this transient insulin resistance results from CLA-induced changes in adipocyte dynamics, ie, older less responsive cells being replaced by younger more metabolically active cells. It should also be noted that in the Zucker fatty rat dietary CLA restores sensitivity and reduces symptoms of diabetes (5, 6).

A related effect, lipodystrophy, was reported in mice fed a diet supplemented with CLA. Like fatty liver, lipodystrophy has not been reported to occur in other species, and it is possible that lipodystrophy is seen in mice because mice are so sensitive to CLA-induced body fat reduction. Increasing the amount of fat in CLA-supplemented diet substantially reduces the lipodystrophy effect (46).

With regard to these seemingly negative effects, it should be noted that dietary CLA significantly extended the life span of NZB/WF1 mice, which are prone to developing lupus erythematosus (47, 48). This finding is consistent with the conclusion that CLA does not induce toxic effects and is important because mice appear to be the most sensitive and responsive known species to the effects of CLA on lipid metabolism (30).

Concern about elevations in oxidative stress and unfavorable changes in blood lipids has arisen from studies by Risserus et al (41) who investigated the effects of CLA in men with metabolic syndrome. They compared a typical high-quality CLA preparation...
consisting of equal amounts of cis\texttext{-}9,11- and cis\texttext{-}10,12-CLA with a supplement that is not commercially available, enriched for cis\texttext{-}10,12-CLA but containing very little cis\texttext{-}9,11-CLA. Riserus et al (41) observed evidence of enhanced lipid peroxidation (measured as urinary isoprostane), enhanced C-reactive protein in serum, and elevated VLDL coupled with reduced HDL. These negative effects were significant relative to placebo for the patients taking the cis\texttext{-}10,12-CLA supplement but were reduced (in some cases below statistical significance) for patients taking the typical commercially available CLA supplement relative to placebo. Interestingly, the apparent cis\texttext{-}10,12-CLA-induced oxidative stress did not result in reduced blood antioxidants but rather was correlated with increased blood vitamin E.

Riserus et al (41) concluded that these findings indicate that CLA could enhance inflammation and cardiovascular disease risk. However, other groups that have studied this issue have concluded that CLA reduces inflammation. For example, pigs fed a commercial mixture of CLA isomers displayed reduced inflammation (17), and dietary CLA not only reduced atherosclerosis in animal models (9,49) but also reduced preestablished atherosclerotic lesions in rabbits (9) and mice (50). Additionally, the researchers of a comprehensive clinical study of the effects on serum lipids of a high-quality CLA preparation consisting of cis\texttext{-}9,11- and cis\texttext{-}10,12-CLA in approximately equal amounts concluded, "The study confirms that some of the cardioprotective effects of CLA that were shown in animal studies are relevant to man" (7).

Riserus et al (41) also reported that the men in their study who were given the cis\texttext{-}10,12-CLA supplement exhibited enhanced insulin resistance. Again, there was no significant difference in this condition between the placebo group and the group receiving the typical commercially available high-quality CLA isomer mixture, a finding that is also consistent with a previous report from Riserus’s group (51). Evidence of enhanced insulin resistance has not been observed in other CLA clinical trials either (7,32,33).

It is well documented that cis\texttext{-}10,12-CLA reduces milk fat. This effect has been most thoroughly studied in cows (52) but is also observed in lactating women who consume CLA supplements (53). The researchers of the latter study (53) concluded that the reduction of milk fat might lead to less energy availability for the nursing infant. However, in a study with rats (54) it was found that the pups nursing dams fed CLA-supplemented diet actually grew to a larger body size. Because very little cis\texttext{-}10,12-CLA carries over to milk, whereas the cis\texttext{-}9,11-CLA isomer is concentrated in milk, it could be concluded that cis\texttext{-}9,11-CLA could be a growth factor for young rats, as it appears to be for young mice (4). Similar findings were obtained in a study with pigs in which the researchers concluded, "Irrespective of the dietary fat supplied in the starter period, piglets reared on the CLA sows had greater final body and warm carcass weights (P <0.01), and greater feed intake (P =0.02) than piglets reared on the [control] sows" (55). It seems likely that in these studies (54,55) nursing animals compensated for reduced calories because of milk fat reduction by consuming more milk.

CONCLUSIONS

In conclusion, the scientific literature on CLA is growing at a phenomenal rate (for current citations to the published scientific literature on CLA, refer to http://www.wisc.edu/fri/clarefs.htm).

The published animal studies and clinical trials indicate the possibility that CLA could be useful in improving human health in a number of areas (Table 1), in particular the reduction of body fat gain (33), immune enhancement against viral antigens (11), and improvement in blood lipids (7). Evidence for efficacy and safety in humans is being steadily strengthened by the results from clinical trials as well as animal toxicology tests. In designing new clinical trials it is crucial to fully appreciate the animal and cell culture database, to ensure that one is asking the appropriate questions.

REFERENCES


