Conjugated linoleic acid: effects on plasma lipids and cardiovascular function

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Conjugated linoleic acid is a collective name for mixtures of several positional and geometric conjugated dienoic isomers of linoleic acid, which have been shown to impact favorably on several biological processes, particularly carcinogenesis. Recent studies have clearly established that the *c*9, *t*11 and *t*10, *c*12 isomers have distinct biological effects. The latter may be of particular importance in affecting blood lipids. Because conjugated linoleic acid has been suggested to be anti-atherogenic, this review is focused on its effects on cardiovascular function. Careful scrutiny of the literature suggests that at present it is premature to assign any beneficial role to conjugated linoleic acid in terms of its ability to impact either blood lipids or atherogenesis. Curr Opin Lipidol 12:31–34.

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Abbreviation

CLA conjugated linoleic acid

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Introduction

Although references to conjugated linoleic acid (CLA) can be traced back to the 1960s [1], current interest in the health benefits of CLA started in the late 1980s, after it was identified as the anti-carcinogenic component present in fried ground beef [2]. Since then, an extensive literature has documented the anticarcinogenic effects of CLA [3,4•,5,6•,7,8]. In addition, there is some evidence that CLA is also anti-atherosclerotic [9,10], has beneficial effects on immune function [11,12], is a growth factor in rats [13], and may play a key role in helping to regulate body fat [14]. Whereas any one of these effects would be considered advantageous, the possibility that CLA affects all of these is fascinating. On top of this, the fact that the richest natural sources of CLA, meat and dairy products, are consumed by people worldwide has tremendous implications for public health. This article will focus on the effects of CLA on plasma lipids and cardiovascular function.

CLA is a collective name for mixtures of several positional and geometric conjugated dienoic isomers of linoleic acid, with the *c*9, *t*11 isomer being particularly important from the standpoint of diet [15]. The *t*10, *c*12 isomer is present in relatively high concentrations in several commercial CLA preparations. *In vivo*, *c*9, *t*12 is formed during the microbial biohydrogenation of linoleic and linolenic acid by a specific enzyme found in ruminant animals [16]. The *c*9, *t*11 and *t*9, *c*11 isomers are preferentially incorporated into various tissue phospholipids [17], and also occur in relatively high concentrations in several uncooked meats [15]. Evidence is mounting that the *c*9, *t*11 and *t*10, *c*12 isomers have distinct biological effects [4•]. It can be anticipated that future research efforts will focus on what each of the various isomers does.

Serum levels of CLA increase in humans consuming foodstuffs rich in CLA [18], with a 19–27% increase reported in individuals consuming a cheddar cheese supplement that provided ~170 mg CLA a day [19]. CLA is known to occur in a wide variety of foods that include ruminant meats, milk, other dairy products, partly hydrogenated vegetable oils and to a much lesser extent certain vegetable oils [15,20,21]. The *c*9, *t*11-isomer accounts for up to 80–90% of the total CLA in meats and dairy products, whereas this isomer accounts for less than 50% of total CLA in seafoods and vegetable oils. Although diet is the major source of CLA in humans, there is little information about typical CLA

consumption patterns. It is only in recent years that the CLA content of foods has become available [15,20]. On the basis of this information, typical US consumption has been estimated (based on the amounts present in meat and dairy products) to be of the order of several hundred milligrams per day [15], whereas a figure of ~400 mg per day has been reported for Germany [22]. As yet there is no systematic database for the CLA content of foods.

Conjugated linoleic acid, plasma lipids and cardiovascular function

Although CLA is suggested to be anti-atherogenic in several review articles, there are as yet only three reports detailing its effects on atherosclerosis. Lee *et al.* [9] fed rabbits semi-synthetic atherogenic diets (14% fat and 1% cholesterol) with or without CLA (~0.5 g/day per rabbit) for 22 weeks. CLA feeding was associated with significant reductions in total cholesterol, LDL-cholesterol and plasma triacylglycerol concentrations. CLA did not affect HDL-cholesterol concentrations *per se*; accordingly the decrease in LDL-cholesterol resulted in a significant reduction in the LDL-cholesterol : HDL-cholesterol ratio. Autopsy data revealed a consistent, albeit non-significant, tendency towards less atherosclerosis in the aortas of the animals fed CLA. In a recent review article, Kritchevsky [6•] provided information (from one of his studies that is as yet unpublished) that feeding 1% CLA as part of a semipurified atherogenic diet (0.2% cholesterol) had a significant beneficial impact on both the progression and regression of atherosclerosis. The effects on atherosclerosis were observed even though plasma lipids were unaffected. Nicolosi *et al.* [10] fed hamsters atherogenic diets for 11 weeks, and investigated three different levels of CLA (0.06, 0.11 and 1.1%). All levels of CLA significantly reduced total cholesterol and VLDL plus LDL-cholesterol concentrations, whereas HDL-cholesterol was not affected. Neither levels of CLA resulted in statistically significant reductions in fatty streak areas (measured in the aortic arch), although combined data from the three CLA-fed groups revealed a significant 26% reduction in fatty streak area compared with control animals. In both the rabbit and hamster studies, a commercial preparation of CLA (present as the free fatty acid) was used, which contained a mixture of CLA isomers.

In contrast to the above studies, Munday *et al.* [23••] recently reported that CLA promoted fatty streak formation in C57BL/6 mice. Animals were fed diets containing 0.5% CLA, 0.25% CLA plus 0.25% linoleic acid or a control diet with 0.5% linoleic acid. All diets contained 1% cholesterol. Although CLA-fed animals had a less atherogenic lipoprotein profile (tendency for lower total cholesterol, higher HDL-cholesterol, resulting in a lower ratio of total to HDL-cholesterol), the total aortic fatty streak area was significantly higher, suggesting that CLA promoted atherogenesis independent of plasma lipids. It is possible that any beneficial effects of CLA in this model were swamped by the very high levels of dietary cholesterol relative to the body weight of the animals. Alternatively, in this particular model, a higher level of CLA might be needed to see a beneficial effect on atherogenesis. The three studies evaluating CLA and atherogenesis, carried out in three different animal models, are thus inconclusive in predicting how CLA will behave in man. As succinctly discussed recently [24], there is at present no evidence in support of the anti-atherogenic effect of CLA.

In addition to the above, several studies have reported on the effects of CLA on plasma lipids. Stangl *et al.* [25•] investigated the effects of CLA on lipoproteins in female pigs. Animals were fed an experimental diet containing 1% CLA (mixture of CLA isomers; approximately 35% *c*9, *t*11 plus *t*9, *c*11, 18% *t*10, *c*12, 5% *t*9, *t*11 and 2% *c*9, *c*11). The α-tocopherol content of both control and test diets was equalized by adding α-tocopherol to the test diet. CLA feeding did not affect total plasma triacylglycerol or total cholesterol concentrations, but significantly increased the LDL-cholesterol : HDL-cholesterol ratio by 26% ($P < 0.04$), reflecting a non-significant increase in LDL-cholesterol. Sugano *et al.* [26] found no difference in serum total cholesterol or HDL-cholesterol in rats fed diets containing 1% CLA.

The above studies of CLA action on plasma lipids used a mixture of isomers and the free fatty acid. The first study utilizing individual isomers of CLA incorporated into triglycerides was recently reported [27••]. In that study, hamsters were fed purified diets (30% from fat, 0.01% cholesterol), in which total CLA represented approximately 5% by weight. Three diets were used, one contained a CLA mix (2.4% *c*9, *t*11 and 2.4% *t*10, *c*12), one was based on the *c*9, *t*11 isomer (4.5% by weight), whereas the other contained the *t*10, *c*12 isomer (4% by weight). The interesting finding was that the CLA mix and the *t*10, *c*12 isomer decreased LDL-cholesterol, decreased VLDL triglyceride and lowered HDL-cholesterol concentrations, whereas the *c*9, *t*11 isomer had no such effect. That study thus seemed to suggest that the *t*10, *c*12 isomer is the key isomer that affects plasma lipid levels.

Mechanism of action of conjugated linoleic acid

Collectively, the above studies do not provide any clear-cut evidence for the effects of CLA on blood lipids or atherogenesis. Although CLA inhibited atherogenesis in the rabbit [9] and hamster [10], it increased atherogenesis in the mouse model [23••]. As far as blood lipids are concerned, LDL-cholesterol was either decreased [9,10,27••], increased [25] or unaffected [23••]. These

disparate effects no doubt reflect the different animal models used, as well as the fact that the isomers utilized were generally not clearly identified. In addition, differences in feeding regimens (e.g. different levels of cholesterol employed) would certainly have been a factor. Regardless of this, the decrease in LDL-cholesterol observed [9,10,27••] is consistent with both decreased apolipoprotein B secretion, observed in Hep G2 cells [28], and decreased intracellular triacylglycerol, observed in mouse adipocytes [29], after treatment with the *t*10, *c*12 CLA isomer. With regard to atherogenesis, there are as yet no convincing data to answer this question emphatically. Originally, CLA was thought to protect LDL from oxidation because it was a more potent antioxidant than α-tocopherol [17]. The antioxidant hypothesis was disproved, because it was found that CLA could not effectively protect membranes from oxidative modification under conditions of metal ion-dependent oxidative stress [30]. Although CLA may not function as a true antioxidant, it has been found that auto-oxidation of CLA produced furan fatty acids, which may protect against oxidant-mediated toxicity [31].

As CLA is incorporated into cell membrane phospholipids [17], it may modify their fluidity and exert its effects by altering intracellular events via one or more signal transduction pathway(s) or eicosanoid synthesis. Because CLA is a polyunsaturated fatty acid, it may compete with linoleic acid in the pathway of eicosanoid synthesis, via the cyclooxygenase or lipoxygenase pathways. A recent study [32] suggested that various isomers of CLA can be precursors for eicosanoid synthesis as they are elongated and desaturated in a manner similar to linoleic acid. It thus seems feasible that these CLA-derived eicosanoids could affect a multitude of pathways involved in lipid metabolism. In this regard, prostaglandin E2 synthesis is known to be decreased by CLA [33,34]. The potential for CLA to affect intracellular lipid metabolism also comes from recent work on the stearoyl coenzyme A desaturase enzyme. The latter is responsible for desaturating palmitic and stearic acids to palmitoleic and oleic acid, respectively . It has been shown that hepatic stearoyl coenzyme A desaturase messenger RNA expression is decreased by CLA [35], and that this downregulation is specific for the *t*10, *c*12 isomer [36••]. In addition, the elegant studies by Moya-Camarena and colleagues [7,37] clearly established that CLA is both a ligand and an activator for the peroxisome proliferator-activated receptor α. This transcription factor modulates gene expression for several enzymes and proteins involved in lipid metabolism, including lipoprotein lipase [38], fatty acid binding protein [39], and acyl coenzyme A oxidase [40]. Furthermore, different CLA isomers have different potencies for peroxisome proliferator-activated receptor α [37].

Conclusion

Although there is a vast database on the anticarcinogenic properties of CLA, knowledge of its effects on lipids, atherogenesis and other aspects of cardiovascular function is still limited, and as exemplified by the data on atherogenesis and blood lipids, there is no clear consensus. However, almost every review article published seems to suggest that CLA inhibits atherogenesis. As elegantly pointed out by Rudel [24], the published data do not permit such a conclusion. After almost a decade of active research, there are no published data on humans or non-human primates. In no species has a consistent, reproducible, dose-dependent effect of CLA been established. Until such data are published, the debate on the involvement of CLA in cardiovascular function and blood lipids will continue. The availability of different CLA isomers should help to resolve some of the questions.

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