Conjugated Linoleic Acid: A Review

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Abstract
Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of the omega-6 essential fatty acid linoleic acid (cis-9, cis-12, octadecadienoic acid). In humans evidence is currently ambiguous as to whether CLA supplementation has a significant effect on body composition. Despite favorable changes in lipid levels in animal models, a beneficial effect in humans has not yet been established. While some of the changes reported are consistent with an improved lipid profile, declines in HDL and increases in lipoprotein (a) have also been observed in some subjects. Available evidence suggests CLA supplementation has no impact on immune system performance in healthy subjects.
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Introduction

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of the omega-6 essential fatty acid linoleic acid (cis-9, cis-12, octadecadienoic acid). The biochemical nomenclature for linoleic acid designates this fatty acid as an 18-carbon ("octa-deca") fatty acid containing two double bonds ("di-en"), specifies the location of the double bonds (the 9 and 12 carbon atoms), and identifies the double bonds as being in a cis-isomeric configuration. This structural configuration results in two single bonds separating the double bonds. CLA is formed when reactions shift the location of one or both of the double bonds of linoleic acid in such a manner that the two double bonds are no longer separated by two single bonds. Unlike linoleic acid, which is a single unique molecule, several dozen different CLA isomers are possible depending on which double bonds are relocated and the resultant isomeric reconfigurations. Figure 1 depicts linoleic acid and several CLA isomers.

Virtually all cis- and trans-isomeric combinations of CLA have been identified in food; however, the most commonly occurring CLA isomer found in the diet is cis-9, trans-11, octadecadienoic acid (c-9, t-11 CLA) (Figure 1). An important distinction is that CLA, unless otherwise specified, should be construed to indicate a mixture of isomers. In other words, CLA is a category, while c-9, t-11 CLA, as an example, refers to a unique molecule. This distinction is important since it appears different isomers might have different activities *in vivo*. Since synthesizing and isolating each unique CLA isomer from vegetable oils is a more difficult and expensive process than generating a mixture of CLA isomers, the majority of research to date

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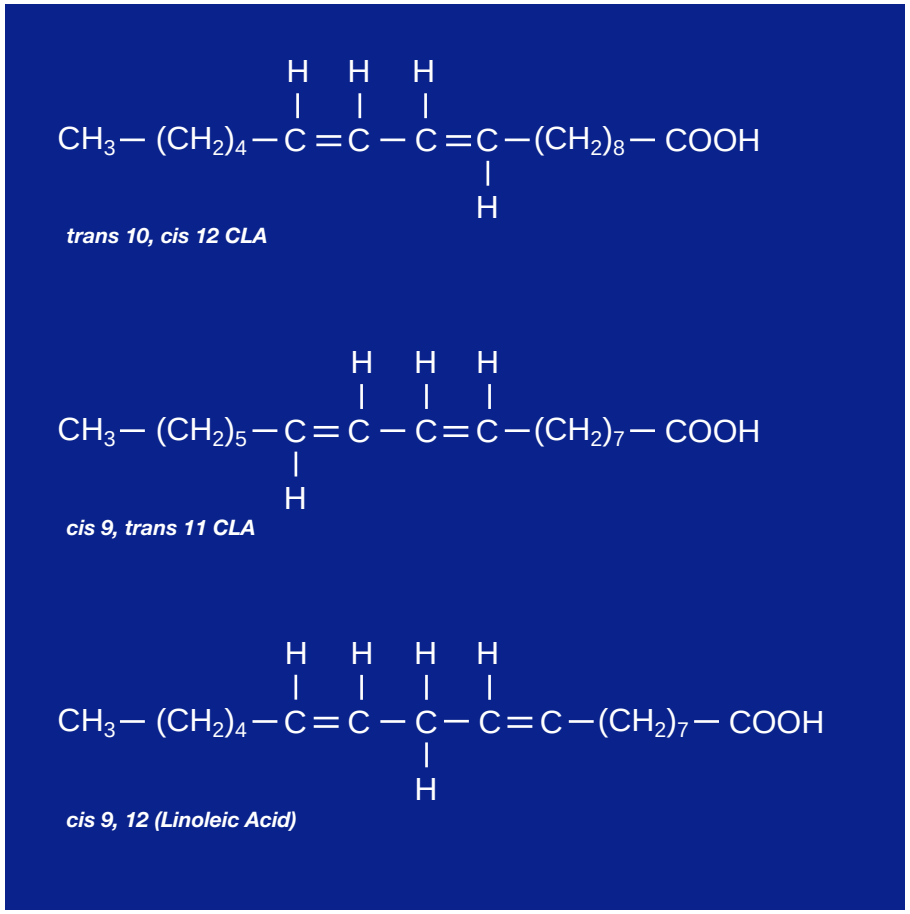
Figure 1. Structure of Linoleic Acid and Common CLA Isomers



has been conducted on mixtures of CLA isomers. The c-9, t-11 CLA and the trans-10, cis-12 octadecadienoic acid (t-10, c-12 CLA) isomers predominate in these mixtures (approximately 85-90%). These two isomers are usually represented in about equal amounts in synthesized CLA, with ten other minor CLA isomers representing the remaining 10-15 percent of these mixtures.[1]

Dietary Exposure to CLA

Exposure to dietary CLA is primarily a result of consumption of dairy products and beef fat. This is because CLA is formed as a result of rumen gut microbial isomerization of dietary linoleic acid and desaturation of oleic acid derivatives. As an example, CLA (primarily the c-9, t-11 CLA isomer) is formed readily by linoleic acid isomerase enzyme activity generated by *Butyrivibrio fibrisolvens*, an endogenous bacteria found in the rumen of cattle and dairy cows.[2] Evidence has also demonstrated the endogenous synthesis of CLA from trans-11, octadecanoic acid occurs subsequent to bacterial delta-9 desaturase enzyme activity in cows. This enzyme inserts a cis-double bond at carbon 9. The result is the formation of the c-9, t-11 CLA isomer. This biochemical pathway is responsible for the majority of CLA found in products created from milk fat of lactating cows fed grain-based diets.[3] As a result of the isomerization and desaturation reactions in cattle, c-9, t-11 CLA is the predominant naturally occurring CLA isomer in the human diet.[3,4]

CLA concentrations in dairy products typically range from 2.9 to 8.92 mg CLA/g fat, of which the c-9, t-11 CLA isomer makes up between 73-93 percent of the total CLA.[5] The conjugated linoleic acid content of cheeses typically ranges from 3.59 to 7.96 mg CLA/g fat. Blue, brie, edam, and Swiss cheeses have been found to have significantly higher CLA content than other cheeses. Sharp cheddar cheeses tend to have higher CLA content than medium cheddar cheeses.[6] CLA content of cultured dairy products typically ranges from 3.82 to 4.66 mg CLA/g fat. Of examined cultured dairy products, cultured buttermilk had the highest content.[6] CLA content of cow's

milk typically ranges from 3.38 to 6.39 mg CLA/g fat;[6] however, significant variation of CLA content of cow's milk products occurs.[7]

When milk from various herds of cows in New York State was examined, CLA levels ranged from 2.4 to 18 mg CLA/g fat. A survey of milk from Canadian creameries uncovered a similar range of CLA levels. This greater than seven-fold variation in CLA levels was attributed primarily to differences in feed practices and dairy farm management.[7] The amount of CLA found in dairy and beef is a direct reflection of the diet the animals are fed. Evidence suggests CLA increases linearly when animals are pasture-fed and decreases when grass intake declines.[8] In one experiment, it was found that cows receiving no supplementary feed and allowed to meet all energy requirements from grazing in pastures had 500-percent more CLA in their milk fat than cows fed a diet consisting exclusively of supplementary feed.[9] CLA content in cows allowed to pasture feed naturally varies throughout the year. Typically, cow's milk produced from late spring through early fall (seasons of rapidly growing green grass) will have almost twice the CLA content of milk produced in winter months. However, no seasonal variation in CLA content is found in dairy cows given the same diet throughout the year.[7]

CLA content of milk fat can be influenced by directly manipulating the type of dietary supplements fed to dairy cows. Supplementing the diet with polyunsaturated oils that contain either linoleic acid (like corn oil or sunflower oil) or linolenic oil (fish oil) increases CLA content of milk fat substantially.[4,9,10] Similar to what is found with respect to grazing, the c-9, t-11 CLA isomer predominates when dairy cows receive diets supplemented with these polyunsaturated oils.[4] Milk fat from cows fed marine algae has also been shown to contain greater concentrations of CLA.[11]

Pharmacokinetics

Unlike ruminating animals, human production of CLA from linoleic acid does not appear to occur to any significant degree. In one experiment, feeding 16 g/day linoleic acid (from 21 g safflower oil) for six weeks resulted in no changes in plasma levels of CLA.[12] The amount of CLA in human adipose tissue is directly related to milk fat intake. The primary isomer that accumulates in human tissue subsequent to milk fat intake is c-9, t-11 CLA. In humans, incorporation of CLA is tissue dependent, with adipose and lung tissues containing the highest concentrations of CLA.[13]

In rats, consumption of CLA-enriched butter results in greater accumulation of CLA in the mammary gland and other tissues when compared with feeding CLA as free fatty acids.[14] This suggests that in rats the pharmacokinetics of CLA varies depending on the form in which CLA is supplied. The majority of commercially available CLA is in the form of free fatty acids and not the triglyceride-bound CLA occurring in food. It is not currently known whether the pharmacokinetics of CLA preparations in humans is influenced by feeding CLA as free fatty acids as opposed to a triglyceride-bound preparation.

In mice, tissue CLA levels decline steadily following the withdrawal of CLA from the diet.[15] Since tissue levels of CLA in humans seem to be a direct reflection of dietary exposure to CLA,[13] it is likely that a similar decline would result in humans subsequent to CLA withdrawal from the diet.

CLA Effect on Body Composition

CLA-feeding has been shown to reduce body fat in several animal models, independent of the type or quantity of dietary fat consumed. Evidence also suggests that feeding different isomers of CLA to animals might have varying effects on weight loss and possibly body composition parameters. Compared with the quantity of research conducted on

weight loss and body composition in animal models, the research on CLA in humans is limited. The available human research is not in complete agreement; however, evidence suggests the possibility that supplementing the diet with CLA might generate favorable changes in body composition in some human subjects.

Animal *In Vivo* Research

AKR/J mice, a strain susceptible to dietary obesity, were fed a high fat diet with or without dietary CLA (1% of diet) for five weeks. Feeding CLA resulted in approximately a 50-percent reduction in adipose tissue weight compared with the mice in the group not receiving CLA. Total body weight was similar between the two groups, suggesting an increase in lean mass as well as a reduction in fat mass in the CLA-supplemented mice.[16] In an independent study, mice fed a high-fat diet and receiving CLA at varying dietary doses (0%, 0.25%, 0.5%, 0.75%, and 1.0%) were observed for a 12-week study period. Body fat was significantly lower and body protein content significantly higher in the mice fed CLA at doses greater than 0.5 percent of the diet.[17] Other experiments on male and female mice of varying genetic strains have shown similar positive changes in body composition subsequent to feeding CLA at dietary concentrations ranging from 0.5-1.0 percent of calories.[15,18-20]

The reductions in adipose tissue in mice subsequent to CLA feeding have been shown to occur in mice fed high-fat diets (45% of energy intake as dietary fat)[16,17,21] and low-fat diets (15% of energy intake as dietary fat).[21] While some experimental evidence suggests the addition of CLA to the diet of mice results in no changes in total energy intake,[16] other experimental evidence has shown that mice fed CLA consume significantly fewer calories.[15] In mice, the changes in body composition appear to be sustained after dietary CLA has been discontinued. Even subsequent to clearance of CLA from tissues, a degree of the improved body composition of mice previously fed CLA seems to be maintained.[16]

CLA might not produce identical results in all animal models. Growing female rats fed diets containing 0.5-percent CLA experienced a modest reduction in adipose tissue mass; however, the reduction was far less than that seen in the majority of studies conducted in mice.[22] It is not clear why these rats were apparently less sensitive to the effects of CLA on adipose tissue.

Female New Zealand white rabbits fed 0.5 g per day CLA actually gained more weight during the first few weeks than rabbits in a control group fed an identical diet supplemented with 0.5 g coconut oil instead of the CLA. At the end of this 22-week study no differences in body weight were seen between CLA-fed and control groups.[23] Since body composition parameters were not measured in this study, it is not possible to determine whether the initial greater weight gain was a result of gains in body fat, lean mass, water weight, or a combination of these factors. It is also not possible to determine whether similar ending body weights in the CLA and control groups were reflective of similar body compositions.

Even in mice, not all studies have shown consistent body composition changes. Although adding CLA to either high- (45% of energy intake as dietary fat) or low- (15% of energy intake as dietary fat) fat diets was shown to result in a reduction of adipose tissue weight ranging from 43-88 percent, reductions in whole body protein weight were also observed.[21]

Evidence suggests that feeding different CLA isomers to animals might have different effects on body composition parameters; however, definitive conclusions cannot currently be drawn based on available information. In one experiment, mice were fed CLA preparations enriched for either the c-9, t-11 CLA isomer or the t-10, c-12 CLA isomer. Favorable changes in body composition as

Table 1. Changes in Body Composition as a Result of 12 Weeks of CLA Feeding

	ΔBody Weight (kg)	ΔBody Fat Mass (kg)	ΔLean Body Mass (kg)
Olive oil 9 g/d	+1.4 +/- 1.9	+1.47 +/- 2.43	-0.05 +/- 2.43
CLA 1.7 g/d	-0.4 +/- 2.6	-1.15 +/- 2.69	+0.87 +/- 1.57
CLA 3.4 g/d	-0.4 +/- 1.7	-1.73 +/- 1.90	+1.26 +/-2.17
CLA 5.1 g/d	-0.1 +/- 0.9	-0.43 +/- 1.74	+0.54 +/- 1.44
CLA 6.8 g/d	-0.8 +/- 2.0	-1.30 +/- 1.46	+0.88 +/- 1.06

Values represent the mean changes and the standard deviation for the group
Δ indicates change. Adapted from Blankson H, Stakkestad JA, Fagertun H, et al. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000;130:2943-2948.

determined by reduced body fat, enhanced whole body water, and enhanced whole body protein were associated only with feeding the t-10, c-12 CLA isomer.[24]

Hamsters were assigned to one of three diet groups to determine whether the c-9, t-11 CLA isomer had the same effect on body composition as a mixture of CLA isomers. All groups of hamsters received a non-purified diet supplemented with 10-percent hydrogenated coconut oil and 0.05-percent cholesterol for six weeks. One group of hamsters had the baseline diet supplemented with a mixture of CLA isomers (1% of the diet). One group had the baseline diet supplemented with the same amount of the c-9, t-11 CLA isomer (0.2% of the diet) as was found in the group fed the isomer mixture. The third group received linoleic acid (0.2% of the diet). After six weeks, the mixture-fed hamsters had lower weight gain even though they had greater food intake than the other two groups.[25] Since this study did not directly measure body composition parameters and focused solely on body weight, it is not possible to draw the conclusion that the c-9, t-11 CLA isomer was ineffective.

Human *In Vivo* Research

Atkinson et al reported no changes in body composition variables among obese subjects given CLA (2.7 g per day) during a six-month, placebo-controlled, randomized, double-blind study.[26] These findings were in contrast to those reported by Blankson et al who reported a positive influence of CLA on body composition variables.[27]

Blankson et al conducted a 12-week, randomized, double-blind study on 60 overweight or obese male and female volunteers (body mass index (BMI) 25-35 kg/m^2). Subjects were divided into five groups. The placebo group received 9 g/day olive oil, and the four treatment groups received different doses of CLA (1.7, 3.4, 5.1 or 6.8 g/day CLA) plus additional olive oil to bring the supplemented fatty acid total to nine grams daily. CLA was provided as a mixture of isomers consisting primarily of relatively equal concentrations of c-9, t-11 and t-10, c-12 isomers. The daily doses of CLA and placebo were divided into three equal doses taken at breakfast, lunch, and dinner. Forty-seven subjects completed the

study. Trends toward decreased body weights and BMI were reported for subjects receiving CLA. A trend toward slight increases in body weight and BMI was found among subjects receiving the placebo. A significant reduction in body fat mass was found in the subjects receiving CLA at the doses of 3.4 and 6.8 g/day when these subjects were compared to subjects receiving placebo. A trend in favor of increased lean body mass was found in all groups receiving CLA; however, this trend only reached significance in the group receiving 6.8 g/day CLA. The greatest changes in body fat mass reduction and lean body mass gain were found among the subjects receiving the 3.4 g daily dose of CLA.[27] Table 1 summarizes the findings from this trial.

The duration of light and intense exercise training was also monitored during this study period. Each CLA-treated group (as an average) increased either the duration of light or intense exercise training or both. The placebo group had an average decrease in duration of both light and heavy training exercise. The researchers suggested that based on the design of this study it was not possible to determine whether the increased lean body mass found in the CLA subjects was a result of the increase in training activities or a consequence of CLA feeding.[27]

A study by Zambell et al found no significant changes in body composition. Seventeen women age 20-41 were confined to a metabolic suite for a 94-day period. All subjects had body weights within 110-120 percent of ideal body weights based on 1983 Metropolitan Life Insurance Company standards. Subjects were fed either CLA (3 g/day as a mixture of CLA isomers) or a sunflower oil placebo beginning on day 30 and continuing for 64 days. Diet and activity levels were held constant throughout the study period. No statistically significant differences were observed between groups in any parameter of body composition measured; however, a non-significant trend toward improved body composition was observed among subjects receiving the CLA supplementation. Although slight increases in fat-free mass were found in both the placebo and CLA group (0.18 +/- 0.43 kg versus 0.09 +/- 0.35 kg, respectively), the increase for the group receiving CLA was greater. Changes in fat mass (0.01 +/- 0.64 kg versus 0.19 +/- 0.53 kg, placebo versus CLA, respectively); percent body fat (0.05 +/- 0.62% versus 0.67 +/- 0.51%, placebo versus CLA, respectively); and body weight (0.48 +/- 0.55 kg versus -0.24 +/- 0.46 kg, placebo versus CLA, respectively), were greater in the CLA group.[28] In this study, no statistical differences in energy expenditure, fat oxidation, or respiratory exchange ratio were observed between groups.[28] Changes in appetite between the supplementation and baseline periods were not observed. Changes in appetite also were not observed when the CLA group was compared with the placebo-fed group.[29]

Mechanisms of Action of CLA

No definitive mechanism has been found to explain the changes in body composition found in animal studies. It is possible CLA might have slightly or even vastly different physiological actions depending on the animal species and perhaps genetic strain of a single animal species being investigated; so, a consistent mechanism might not exist.

In vitro evidence from mice suggests CLA might impact body composition in part by increasing lipolysis and beta oxidation of fatty acids, and reducing the deposition of fatty acids in adipose tissue.[18] *In vitro* evidence also suggests different isomers of CLA might have different effects on body composition. In cultured adipocytes from mice, the t-10, c-12 CLA isomer stimulated lipolysis, whereas, the c-9, t-11 and t-9, t-11 CLA isomers were ineffective under these *in vitro* conditions.[24]

In vivo, dietary CLA has been shown to increase energy expenditure in mice. The increase in energy expenditure was sufficient

to account for lower body fat stores found in CLA-supplemented mice.[26] These animal findings are in contrast to those of Zambell et al conducted in humans. In the female subjects they investigated, no changes in energy expenditure were observed subsequent to 64 days of supplementation with 3 g/day CLA.[28]

In a study of female mice, fat-mass decrease by CLA was mainly due to apoptosis of adipose tissue cells.[19] In rats, the reduced fat pad size was due to smaller adipocyte size rather than a reduced cell number.[22] This suggests that in rats CLA did not cause destruction of fat cells. In this same study, the rats fed CLA had an increase in fatty acid synthesis in liver and adipose tissue. An increase in endogenous fatty acid synthesis has also been reported in mice fed CLA.[26] Increased activity of fatty acid synthesis would not generally be considered a favorable physiological alteration with respect to obesity management, since it indicates an up-regulation of endogenous pathways of fatty acid creation. It is not clear what physiological factors helped overcome this increase in endogenous fatty acid synthesis, since in both animal experiments CLA supplementation resulted in a decrease in adipose tissue weight.

Effect of CLA on Insulin Resistance

While CLA has received a great deal of attention as a supplement that might favorably modify body composition, its potentially adverse impact on insulin resistance has not received equal attention. If CLA has the effect on insulin levels and activity in human patient populations that it has had in some experimental animal studies, such action would be potentially undesirable.

In Vivo Research in Animals

In several animal studies, dietary CLA has apparently given rise to increased insulin levels.[16,17,19,30,33] In AKR/J mice (a strain susceptible to dietary obesity), adding CLA as one percent of dietary calories resulted in a nearly two-fold increase in plasma insulin levels. In these mice there was also a trend toward higher blood glucose levels.[16] The combination of both higher circulating insulin and glucose levels suggests impairment in the ability of insulin to dispose of glucose in tissues, a finding consistent with the development of an insulin resistant state. Even though these mice had a reduction in adipose tissue weight, the development of an insulin resistant state would not be construed as a beneficial metabolic change.

In female mice fed standard semi-purified diets (10% of total energy as fat) with or without CLA (1%), fat mass decrease in these animals was attributed to apoptosis. Tumor necrosis factor-alpha (TNF-α) levels increased 12-fold in isolated adipocytes from CLA-fed mice compared with control mice.[19] TNF-α increases have been reported to be associated with insulin resistance and abdominal obesity in humans.[31] In this study's mice, the decrease in fat mass subsequent to CLA supplementation resulted in a state resembling lipoatrophic diabetes; i.e., ablation of brown adipose tissue, a marked reduction of white adipose tissue, marked hepatomegaly, and marked insulin resistance.[19] Other studies on mice fed CLA have also found increases in circulating insulin levels, in liver weight,[17,30] and in TNF-α.[32]

The effect of CLA administration on insulin levels, glucose tolerance, and glucose homeostasis was investigated in male Zucker diabetic rats (an animal model of type 2 diabetes). When CLA-fed rats were compared with lard-fed rats, a reduction in insulin levels was seen in the CLA group; however, the CLA-fed rats remained markedly hyperinsulinemic. An improvement in glucose disposal and a more rapid return to baseline glucose levels subsequent to a glucose infusion was also observed in the CLA-fed rats.[33] While CLA feeding was superior to lard feeding in these genetically susceptible rats, it is important to note that these rats developed a substantial degree

of insulin resistance as indicated by insulin levels approximately 10.5 times higher than levels found in lean controls.[33]

Feeding CLA to adult female pigs was reported to increase fasting insulin levels. One group of female pigs was fed a diet containing one-percent CLA. These animals were compared with another group fed an isoenergetically identical diet containing no CLA. After six weeks, no differences in body weights were found between the two groups; however, the CLA-treated pigs exhibited a 37-percent higher concentration of fasting serum insulin.[34]

In Vivo Human Research

Insulin levels have been monitored only in one human trial of CLA supplementation. Among women (ages 20-41 years) who were confined to a metabolic suite for a 94-day period, 3 g/day CLA resulted in an insignificant trend toward increased mean insulin levels toward the end of the supplementation period. CLA supplementation did not change blood glucose levels during the treatment period.[29]

Effect of CLA on Cardiovascular Disease

Although CLA has received attention suggesting it might benefit cardiovascular health,[35] there is currently no evidence to support the hypothesis that CLA protects against atherogenesis in humans. Even in animal studies, adding CLA to a diet that would otherwise produce atherosclerosis resulted in mixed results depending on the type of animal and, even within animals of the same species, the genetic strain being studied. Similarly, the ability of CLA administration to positively modify lipid levels has not occurred in all animal models. The one available human trial did not demonstrate clinically significant improvement in lipid levels and no efforts were made to directly monitor atherosclerosis.

In Vivo Research in Animals

Atherosclerosis can be induced in New Zealand white rabbits fed a semi-purified diet containing 0.1-0.2-percent cholesterol. Supplementing this diet with CLA at a level as low as 0.1 percent can inhibit atherosclerosis. Even among the rabbits with established atherosclerosis, adding CLA to the semi-purified diet seems to be beneficial. Adding one-percent CLA to the diet for 90 days resulted in an average regression of established atherosclerosis of 30 percent.[36]

CLA supplementation in conjunction with an atherosclerotic diet fed to rabbits showed similar beneficial effect in retarding atherosclerosis in a second study. The diet contained 14-percent fat and 0.1-percent cholesterol. CLA as 0.5 percent of the diet was fed to 50 percent of the rabbits. Lipid levels among the CLA-supplemented rabbits were considered to be more favorable, consisting of lower triglycerides and low density lipoprotein (LDL) cholesterol levels. Examination of the aortas of CLA-fed rabbits showed less atherosclerosis.[23]

Hamsters fed CLA-containing diets (0.06-, 0.11-, or 1.1% CLA) collectively had significantly reduced levels of plasma total cholesterol, very low density lipoprotein (VLDL) and LDL cholesterol, and triglycerides. No effects were seen on high density lipoprotein (HDL) cholesterol levels. Plasma tocopherol/total cholesterol ratios determined from plasma pools for CLA supplemented animals were significantly increased (86% greater in animals fed 1.1% of diet as CLA). Since no additional tocopherol was contained in the diets of the animals fed CLA, these results suggest CLA had a tocopherol-sparing effect. Morphological assessment revealed less early atherosclerosis in the CLA-supplemented animals.[37] Other studies have shown similar favorable modifications in lipid profiles subsequent to CLA-feeding in hamsters.[25,39]

Table 2. Changes in Lipid Levels as a Result of 12 Weeks of CLA Feeding

	ΔLp (a) mg/L	ΔHDL mmol/L	ΔLDL mmol/L	Δtriglycerides mmol/L
Olive oil 9 g/d	+2 +/- 74.9	-0.1 +/- 0.2	-0.2 +/- 0.8	+0.7 +/- 0.3
CLA 1.7 g/d	-2 +/-45.9	-0.1 +/- 0.1	-0.3 +/- 0.4	+0.9 +/- 0.4
CLA 3.4 g/d	+13 +/- 34.1	-0.1 +/- 0.0	-0.3 +/- 0.3	+0.0 +/-0.5
CLA 5.1 g/d	+2 +/- 49.1	-0.1 +/- 0.1	-0.1 +/- 0.7	+0.04 +/- 0.6
CLA 6.8 g/d	+22 +/- 41.1	-0.2 +/- 0.2	-0.1 +/- 0.5	+0.04 +/- 0.3

Values represent the mean changes and the standard deviation for the group
Δ indicates change. Adapted from Blankson H, Stakkestad JA, Fagertun H, et al. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000;130:2943-2948.

However, CLA does not appear to produce identical results in all animal models of atherosclerosis. Serum of adult female CLA-treated pigs (1% of diet) showed a trend toward increased levels of triglycerides and VLDL and LDL cholesterol, without distinct changes in the HDL fraction. The LDL:HDL cholesterol ratio was significantly increased by feeding CLA to these animals.[34]

In C57BL/6 mice, CLA (0.5% of the diet) was substituted for linoleic acid in the diet. This strain of mice is genetically susceptible to the development of fatty streaks in the intima of the aortic sinus when placed on a diet similar to the control diet used in the study. In these mice, CLA addition to the diet resulted in a lipid profile considered less atherogenic (lower triglycerides, and a higher serum HDL:total cholesterol ratio). Despite the more favorable serum lipid profile, CLA supplemented animals demonstrated a greater degree of development of fatty streaks in aortic tissue than did mice fed the control diet.[38] Since in this study the lipid profile was positively modified and yet atherosclerosis was actually promoted, it might not be prudent to assume that an improvement in lipid profiles subsequent to CLA-feeding in other animal models actually means the risk of atherosclerosis has been lowered.

In Vivo Human Research

In a study of human subjects with BMIs between 25-35 kg/m^2, statistically significant reductions in LDL, HDL, and total cholesterol were found in all groups receiving CLA; however, these changes were not large enough to be clinically significant. An increase in lipoprotein (a) was found in the groups receiving 3.4 grams or more of CLA per day. The average increase during the 12-week trial in the group receiving 3.4 grams per day of CLA was 13 +/- 34.1 mg/L. The average increase during the 12-week trial in the group receiving 6.8 grams per day of CLA was 22 +/- 41.1 mg/L. Data are summarized in Table 2.[27]

Effect of CLA on Cancer

The anticancer potential of CLA has received a great deal of research attention in both *in vitro* and animal models.

In Vitro Research

Several studies have demonstrated *in vitro* anticancer activity for CLA. CLA inhibited the proliferation of human hepatoma cell lines.[39] *In vitro*, CLA has shown inhibitory effects on lung adenocarcinoma cell lines (A-427, SK-LU-1, A549) and one human glioblastoma cell line. Unlike CLA, linoleic acid had no ability to inhibit growth of these cancer cell lines.[40] CLA inhibited proliferation of estrogen receptor-positive MCF-7 cells; however, it was ineffective in preventing proliferation of estrogen receptor-negative MDA-MB-231 cells.[41]

While CLA in the form of free fatty acids exerts an *in vitro* antiproliferative effect, milk fat enriched with CLA appears to have even greater *in vitro* activity. Bovine milk fat enriched with CLA was more effective in inhibiting human MCF-7 breast cancer cells than were isolated CLA isomers. Incubation of cells with bovine milk fat enriched with CLA decreased viable cell numbers by up to 90 percent. Incubation with a mixture of CLA isomers or with the c-9, t-11 CLA isomer resulted in a 60-percent decrease. Incubation with the t-10, c-12 CLA isomer caused only a 15-percent decrease in cell numbers under similar conditions. In contrast to the results obtained with the various forms of CLA, incubation with linoleic acid resulted in a 25-percent increase in cell proliferation.[42]

In Vivo Animal Research

In animal experiments, CLA has demonstrated anticancer activity and antimetastatic activity. Dietary CLA has anticancer activity in animal models of prostate[43] and colon[44] cancer; however, the majority of animal cancer research has studied the effects of CLA administration on mammary cancer.

A DU-145 human prostatic carcinoma cell line was injected into severe combined immunodeficient (SCID) mice. The mice were divided into three groups and tumor growth and metastasis were observed. One group was placed on a standard diet. The two remaining groups had a standard diet supplemented with either linoleic acid or CLA at one percent of the diet. Supplementation was started two weeks prior to subcutaneous inoculation with the DU-145 cells and was continued for a period of 14 weeks. The mice receiving CLA had smaller local tumors and a reduction in lung metastasis compared to the animals on both the standard diet and the linoleic acid supplemented diet.[43]

F344 rats were exposed to the carcinogen 2-amino-3-methylimidazol[4,5-f] quinolone in order to induce colon carcinogenesis. Controls and CLA treated rats (at 0.5% of the diet) were exposed to the carcinogen during weeks 3 and 4 of the study period. After week 16 the rats were killed in order to quantify aberrant crypt foci (ACF). While CLA administration had no impact on the size of ACF, the number of ACF was significantly reduced in the CLA group (1.1 +/- 1.3) when compared with the controls (4.3 +/- 2.4).[44]

The timing during the life cycle when CLA is added to the diet appears to exert a profound effect on protection against carcinogens that can initiate mammary tumor formation in mice and rats. It appears the critical time period for dietary exposure to CLA might be prior to mammary gland maturation. Several studies have found that dietary exposure to CLA between the time of weaning and mammary gland maturation has a protective effect against development of tumors due to exposure to carcinogens.

Feeding female rats a diet containing one-percent CLA from weaning until 50 days of age (corresponding with mammary gland maturity) resulted in a significant degree of

protection against cancer formation due to dimethylbenz[a]anthracene (DMBA) exposure. In this study, no added protection against cancer formation was gained when the animals fed CLA from weaning to day 50 were maintained on a diet containing CLA after carcinogen exposure.[45]

In another study, female rats received no dietary CLA from weaning through 50 days of age (corresponding to mammary gland maturity). At day 50, rats were exposed to DMBA and CLA at one percent of the diet was initiated in all rats exposed to the carcinogen. CLA supplementation of the diet was discontinued at four weeks or eight weeks post-carcinogen exposure in some rats, and was continued until the end of the research experiment in other rats (20 weeks). Significant cancer protection was found only in the rats receiving CLA for the entire 20 weeks. As soon as CLA feeding was discontinued, protection against carcinogen-induced cancer formation was lost.[46]

When female rats were fed a diet containing one-percent CLA between early post-weaning and a period analogous to puberty (from 21 to 42 days of age), tumor formation as a result of methylnitrosourea (MNU) administration at 56 days of age was substantially reduced. The rats required no additional CLA supplementation during or after MNU exposure to maintain protection against mammary tumor initiation. In rats not exposed to dietary CLA during the post-weaning to pubertal period, tumor formation subsequent to MNU exposure was maximally prevented only if CLA was continuously supplemented starting after the MNU exposure. If CLA was withdrawn at any point after the MNU exposure in the animals not provided CLA during the period when breast tissue was maturing, tumor inhibition appeared to be lost.[47]

Temporary addition of CLA to the diet subsequent to mammary gland maturation did not provide protection against transplanted metastatic murine mammary tumors in female Balb/c mice. Eight-week-old female mice were fed diets containing 0.1-, 0.3- or 0.9-percent CLA for two weeks prior to transplantation of tumor cells into the mammary gland. Dietary CLA did not impact mammary tumor latency and provided no protection against tumor incidence.[48] In a study of SCID mice, continuous CLA administration (1% of diet), beginning two weeks prior to inoculation of human breast adenocarcinoma cells and continuing throughout the study time period, inhibited local tumor growth and tumor metastasis to lungs, peripheral blood, and bone marrow.[49] These studies taken together suggest that in mice, similar to results reported in female rats, continuous CLA feeding protected against tumor formation.

Addition of CLA to the diet of mice has shown antimetastatic effects in a murine mammary cancer model. Mice were fed a diet consisting of 20-percent fat. Diets were supplemented with either no CLA or 0.1-, 0.5-, or 1.0-percent CLA. Latency, metastasis, and pulmonary tumor burden of transplantable murine mammary tumors were measured. Compared with mice fed a diet containing no CLA, the latency of tumors in mice receiving CLA was significantly increased. Metastasis, as indicated by pulmonary tumor burden, was decreased in mice receiving CLA in the diet. Tumor burden was decreased proportionately with the increasing concentrations of dietary CLA, suggesting a dose-dependent response.[50]

Experimental results suggest a partial selectivity of uptake and accumulation of the c-9, t-11 CLA isomer in the mammary gland of rats. Since c-9, t-11 CLA appears to have greater *in vitro* antiproliferative ability,[42] and since it accumulates to a greater degree in rat mammary tissue, it has been suggested this isomer might be the most important for observed anticancer effects.[14,42]

It appears the dose response to CLA is maximized at a dietary concentration of approximately one percent, and no added protection against mammary carcinogenesis is

gained by increased concentrations of dietary CLA.[52] It also appears CLA prevents mammary cancer development subsequent to carcinogen exposure irrespective of the quantity (from 2-20% of the diet) and type (whether linoleic acid containing vegetable oils, arachidonic acid containing lard, or a combination of both) of other fats found in the diet.[51,52]

Anticancer Mechanisms

In vitro research has suggested CLA's anticancer activity might be partially a result of CLA-inducing lipid peroxidation; however, the evidence for this mechanism is currently inconclusive and it is unlikely that lipid peroxidation is the sole mechanism of action even *in vitro*.[40,41,43] To further weaken the hypothesis that CLA exerts anticancer effects as a result of lipid peroxidation, *in vivo* results indicate dietary CLA does not increase lipid peroxidation in mice with transplanted metastatic murine mammary tumors.[48] *In vivo* evidence has also indicated that adding CLA to the diet of female rats exposed to carcinogens results in lower levels of mammary tissue malondialdehyde (an end product of lipid peroxidation), suggesting the potential for some degree of *in vivo* antioxidant activity.[52] Currently no definitive conclusions can be made; however, it appears unlikely that the mechanism of action is a result of increased lipid peroxidation in target tissue.

Evidence suggests a degree of CLA's activity might be a result of modifying eicosanoid production. Feeding CLA to mice resulted in a decrease in arachidonic acid production.[53,54] Dietary CLA resulted in a dose-dependent trend toward a reduction in the release of leukotriene B4 (LTB4) and a reduction of serum PGE2 levels.[55] Dietary CLA also reduced PGE2 synthesis approximately two-fold in mice treated topically with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate.[56]

CLA might have some effect on the estrogen-mediated mitogenic pathway. *In vitro* evidence suggests a higher percentage of estrogen receptor-positive MCF-7 cells treated with CLA remained in the G0/G1 phase as compared to controls and those treated with linoleic acid. The effect of CLA on these cells was only temporary and was reversed when CLA was withdrawn from the media.[42]

In vivo evidence suggests an ability of CLA to induce apoptosis, suggesting that some of CLA's ability to decrease tumor mass might be a result of inducing programmed cell death.[57]

It is unlikely that CLA's ability to protect rats and mice against cancer is directly related to immune system activation. In two studies, feeding CLA to SCID mice resulted in protection against cancer formation and metastasis via mechanisms independent of the host immune system.[44,49] Available evidence suggests CLA supplementation (3.9 g/day for 64 days) has no impact on immune system parameters in healthy women. No changes were observed after supplementation in the number of circulating white blood cells, granulocytes, monocytes, lymphocytes, circulating B-cells, total T-cells, suppressor and helper T-cells, NK cells, delayed-type hypersensitivity response, and serum antibody titer response to influenza vaccine.[58]

Toxicology

Rat toxicity data indicate that CLA intake as 1.5 percent of the diet for 36 weeks results in no histopathological damage to organs and no hematological abnormalities.[59] However, CLA at one percent of the diet has resulted in hepatomegaly in some mice.[17,19,30] When CLA was added to the diet of autoimmune-prone NZB/W F1 mice, onset of proteinuria was accelerated and the CLA-fed mice had slightly earlier mortality than control fed mice. No effect of dietary CLA was observed on anti-DNA antibody production.[60]

Dosage and Side Effects

A dose of greater than 3 grams per day of CLA appeared to produce the most favorable results in altering body composition in the one positive trial. Since most commercially available CLA is in the free fatty acid form (as opposed to the more expensive triglyceride-bound form), manufacturers of CLA products often recommend that CLA be supplemented in conjunction with food products containing milk fats. It is not currently known whether this recommendation influences CLA absorption and tissue distribution.

Based on preliminary animal and human data, monitoring insulin, glucose levels, and insulin-mediated glucose disposal, as well as serum lipids to include HDL and Lp(a) fractions might be warranted for subjects chronically supplementing CLA. Adverse effects reported after CLA administration in human subjects include gastrointestinal complaints and fatigue.[27]

Discussion

In humans evidence is currently ambiguous whether CLA supplementation has any significant effect on body composition. It is possible that factors such as genetics, current state of health, degree of obesity, and activity levels might influence the impact that CLA supplementation has on altering body composition. Additional research is required to clarify whether CLA supplementation has a role in improving body composition and which subjects, if any, are likely to gain benefits.

The generation of insulin-resistant states and hepatomegaly in some animal experimental models, coupled with the trend toward increased insulin levels in one human study, are areas of potential concern. If long-term supplementation creates or worsens insulin resistance in humans, the clinical utility of this supplement in weight management, whether or not it generates temporary weight loss, should be questioned. Since genetics, activity levels, lifestyle habits, nutritional status, and dietary factors interact in the development of insulin resistance, there is a great deal of potential for inter-individual variation. Until more information is available it seems prudent to monitor glycemic parameters and possibly liver enzymes in subjects choosing to supplement the diet with CLA. It also seems prudent to withhold supplementation from patients with liver disease, insulin resistance, or type 2 diabetes until safety is demonstrated.

While CLA has generated positive changes in lipid parameters in some animal models, in one animal experiment an increase in fatty acid deposition in aortic tissue was noted even though lipid changes were suggestive of lower risk for atherosclerosis. CLA appears to influence lipid levels in humans. Along with a reduction in total and LDL cholesterol, reductions in HDL and increases in Lp(a) have been reported. This suggests some lipid fractions improved while others worsened. Since changes in lipid levels in one animal model did not correspond with atherosclerosis formation, and since its overall impact on lipid fractions in humans is ambiguous, it is impossible to predict the effect of long-term CLA supplementation on cardiovascular health. Whether CLA protects against, promotes, or has no effect on atherosclerosis and cardiovascular disease risk in humans requires further investigation.

CLA appears to have direct anticancer activity in rodent models of cancer. Supplementation has been shown to decrease tumor burden and metastasis in experimental models of transplanted tumors. CLA administration also appears to inhibit the initiation of mammary cancer subsequent to carcinogen exposure in rodent models. A critical time period of dietary exposure to CLA may exist since feeding CLA in the time period prior to mammary gland maturation seems to provide a lasting degree of protection. If dietary CLA is not provided during this developmental time

period, continuous administration of CLA post-carcinogen exposure appears to be required to provide a similar degree of protection against carcinogen-induced cancer formation. Insufficient epidemiological data exist to determine if CLA plays any similar role in human breast cancer risk. The role of CLA in cancer risk and treatment warrants further investigation.

CLA appeared to accelerate some autoimmune processes in autoimmune-prone mice. Available evidence suggests CLA supplementation has no impact on immune parameters in healthy female subjects; however, it is not known whether identical results would be found in individuals with autoimmune conditions. Whether CLA would have any benefit or detriment to immune performance in elderly, immune compromised, or individuals with autoimmune processes requires clarification.

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