Conjugated Linoleic Acid and Dietary Beef

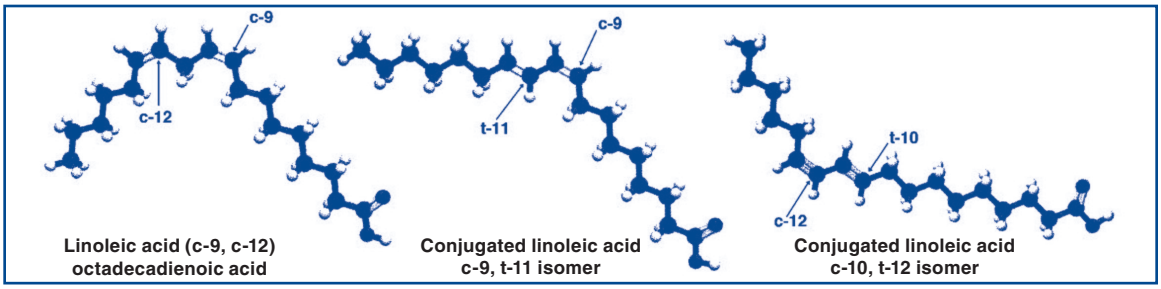
BEEF FACTS
Human Nutrition Research

Conjugated linoleic acid (CLA), a naturally occurring *trans* fat, is commonly found in ruminant animal foods such as beef, lamb, and dairy products (e.g., milk and cheese). Over the past two decades a wealth of research, mainly from *in vitro* and experimental animal studies and limited human investigations, has examined potential health benefits of CLA. This research on the biological functions and health benefits of CLA dates back to the 1980s when scientists at the University of Wisconsin observed that an anti-carcinogenic compound (later identified as CLA) isolated from grilled ground beef inhibited chemically induced skin cancer in mice.[1] Since then, numerous studies have investigated the effects of CLA on cancer, cardiovascular disease, body composition, and other conditions (e.g., insulin resistance, immune function, bone health). The physiological and health effects of CLA are reviewed in several recent publications.[2-8] Also, a listing of the scientific literature on CLA since the 1980s can be found by logging onto *www.wisc.edu/fri/clarefs.htm*.

Structure of CLAs

CLA is a collective term used to describe a mixture of positional and geometric isomers (forms) of linoleic acid, an essential fatty acid. Although 28 different CLA isomers have been identified and it is possible that a number of these isomers have beneficial biological activity, all of the known physiological effects of CLA to date are attributed to two isomers. These are the *cis*-9, *trans*-11 isomer (*c*9, *t*11 CLA, also called rumenic acid), which accounts for 72 to 94% of total CLA in foods from ruminant animals, and the *trans*-10, *cis*-12 isomer (*t*10, *c*12 CLA), which is found in minor amounts in foods from ruminant animals.[6,8-10] Fritsche and coworkers identified 14 CLA isomers in beef fat.[10] The *c*9, *t*11 isomer was the most predominant isomer (72%), followed by the t7, *c*9 isomer (7%), with minor amounts of other CLA isomers such as *t*10, *c*12.[10] Structures of the two most studied, biologically active CLA isomers - *c*9, *t*11 and *t*10, *c*12 - compared with that of the parent linoleic acid are illustrated in Figure 1. Most studies have used synthetic CLA isomer mixtures consisting of *c*9, *t*11 and *t*10, *c*12 CLA isomers in equal proportions (1:1), with other isomers as minor components. The commercial availability of these individual isomers has also led to investigations of their separate, unique roles in health-related disorders. Both isomers have been shown to exhibit significant biological activities, which may be similar or opposite.[8,11]

Figure 1. Chemical Structures of Linoleic Acid and Two Isomers of Conjugated Linoleic Acid (CLA)*



** Adapted from: Steinhart, C. Conjugated Linoleic Acid. The good news about animal fat. J. Chem. Educ. 73: A302, 1996.*

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Origins and Sources of CLA

Origins

CLA is produced naturally in the rumen of ruminant animals by fermentative bacteria, which isomerizes linoleic acid into CLA. Ruminants also synthesize CLA from *trans*-11-18:1 (vaccenic acid), the predominant *trans* monounsaturated fatty acid of animal tissue fat, by way of the enzyme delta-9-desaturase.[9,11] This endogenous synthesis from vaccenic acid is speculated to be the major source of *c*9, *t*11 CLA in the body fat of cattle.[12] Accumulating evidence indicates that naturally occurring *trans* fats (e.g., vaccenic acid, CLA) are beneficial to health.[2,3,8,13-15] Evidence indicates that *trans* fats from ruminant food sources (e.g., meat, milk) are not associated with increased risk of coronary heart disease, whereas *trans* fats formed during the processing of vegetable oils (i.e., man-made or synthetic *trans* fats) are linked to increased risk of this disease.[14,15] The U.S. Food and Drug Administration, recognizing these differences in *trans* fats and the potential beneficial health effects of CLA, excludes CLA from its nutrition labeling regulations requiring disclosure of the level of *trans* fats in foods.[16]

Sources

Representative and relative concentrations of CLA and the proportion of *c*9, *t*11 CLA in a variety of foods are summarized in Table 1.[17] CLA concentrations are highest in foods from ruminants (beef, lamb, dairy products).[9,11,17] Seafood, pork, most poultry products and vegetable oils are not notable sources of CLA.[11,17] The average CLA content in meat products of ruminant origin is reported to be 0.46% of fat (range 0.12 to 1.20%), whereas the CLA in meats of non-ruminant origin averages 0.16% of fat (0.06 to 0.25%).[9] Research has identified foods such as white button mushrooms[18] and pomegranate seed oil[19] as natural food sources of CLA.

The total CLA content of specific foods may vary widely.[9] For example, the total CLA content of beef varies from 0.17 to 1.35% of fat.[9] This wide range is related to the type of feed offered, breed differences, and management strategies used to raise cattle.[9,20] Grazing beef steers on pasture or increasing the amount of forage (grass or legume hay) in the diet has been shown to increase the CLA content in the fat of cattle. Also, supplementing high-grain diets of beef cattle with oils (e.g., soybean oil, linseed oil, sunflower oil) may increase the CLA content of beef.[9,12] Because grazing animals on pasture substantially increases the CLA as a proportion of total fatty acids, but total fat content in the product is reduced, the increase in CLA content should be evaluated on total CLA available in edible fat, rather than in concentrations in raw meat. Breeds of cattle that deposit high amounts of fat in muscle will provide a higher amount of CLA.[20] CLA in meat is stable under normal cooking and storage conditions.[9]

Table 1. Representative/Relative Concentrations of CLA in Uncooked Foods [adapted from Chin et al. (17)].

Food	Total CLA (mg/g fat)	c9, tll-isomer (%)
Meat		
Fresh ground beef	4.3	85
Beef round	2.9	79
Beef frank	3.3	83
Beef smoked sausage	3.8	84
Veal	2.7	84
Lamb	5.6	92
Pork	0.6	82
Poultry		
Chicken	0.9	84
Fresh ground turkey	2.5	76
Seafood		
Salmon	0.3	n.d.*
Lake trout	0.5	n.d.
Shrimp	0.6	n.d.
Dairy Products		
Homogenized milk	5.5	92
Butter	4.7	88
Sour cream	4.6	90
Plain yogurt	4.8	84
Ice cream	3.6	86
Sharp cheddar cheese	3.6	93
Mozzarella cheese	4.9	95
Colby cheese	6.1	92
Cottage cheese	4.5	83
Reduced fat swiss	6.7	90
Am. Processed cheese	5.0	93
Cheez whiz™	5.0	92
Vegetable Oils		
Safflower	0.7	44
Sunflower	0.4	38
Canola	0.5	44
Corn	0.2	39

** n.d. = not detectable*

Dietary CLA Intake of Humans

Current measures of usual or actual dietary intakes of CLA are very limited, most being only estimates. Average estimated CLA intake in U.S. adults is 0.2 g/day,[21] whereas in some other countries such as Germany where the population consumes more energy from ruminant fat, CLA intake is much higher (e.g., about 0.4 g/day).[22] In a small study of free-living adults in Canada, average intake of *c*9, *t*11 CLA (rumenic acid) was about 0.1 g/day (range of 0.02-0.17 g/day).[23]

As shown in Table 2, intakes of total CLA and rumenic acid (i.e., the predominant, biologically active isomer of CLA found in beef) by U.S. adults vary widely. Factors such as the amount, composition (i.e., fat content), and frequency of intake of foods of ruminant origin, as well as the methodology used to estimate food intake influence

Table 2. Estimated CLA Intake by U.S. Adults [adapted from Ritzenthaler et al.[21]].

Total CLA (mg/day)		Rumenic Acid (mg/day)		Methodology
Men	Women	Men	Women	
212 (0-454)*	151 (0-520)	193 (0-439)	140 (0-500)	3-day food duplicate
176 (3-486)	104 (1-399)	133 (1-358)	79 (1-336)	3-day dietary records
197 (0-516)	93 (0-300)	151 (0-412)	72 (0-223)	Food frequency questionnaire

** mean (range)*

CLA intake. With respect to methodology, intakes of total CLA and rumenic acid estimated by three-day dietary records and a semi-quantitative food frequency questionnaire are shown to be significantly lower than those estimated by three-day food duplicates.[21] Also, intakes of CLA and rumenic acid are higher in men than in women, presumably because of men's higher intake of fat from meat such as beef and dairy products.[21,22]

Ruminant products are by far the major contributor of CLA in the diet.[6,11,21] When CLA intake of U.S. adults was estimated by three-day dietary records, it was found that beef provided 32% and dairy foods provided 60% of the intake of CLA.[21] Because ruminant products contain two-fold or more vaccenic acid (the predominant *trans* monounsaturated fatty acid in ruminant fat) than CLA and ~20% of this vaccenic acid is converted endogenously in humans to CLA[22], it is estimated that the effective physiological dose of CLA is much higher (i.e., CLA intake times 1.4).[11] In humans, dietary vaccenic acid can be converted endogenously to *c*9, *t*11 CLA by tissue delta 9-desaturase.[24,25]

Potential Health Benefits of CLA

Cancer

In vitro and experimental animal studies support an anti-carcinogenic effect of CLA, both *c*9, *t*11 and *t*10, *c*12 isomers, at different sites including the mammary gland, colon, prostate, skin, and forestomach.[6,8,26,27] In a variety of human cancer cell lines, CLA has been shown to reduce the growth of cancer cells, whereas linoleic acid has variable effects ranging from inhibition of tumor growth to promotion depending on cell type and degree of malignancy.[6,8,28] In experimental animals, CLA has been demonstrated to inhibit the initiation, progression, and metastasis (spread) of chemically-induced cancers.[6,8,29]

The anti-carcinogenic effect of CLA is most impressive in studies of mammary cancer. In a series of investigations in laboratory rats, Ip and coworkers demonstrated that CLA decreased the incidence and number of chemically induced mammary tumors at various stages of carcinogenesis at relatively low concentrations.[27,30-33] In these studies, CLA inhibited cancer in a dose-dependent manner at levels of 1% (by weight) CLA and below, with no further beneficial effect at levels above 1%.[32] In rats fed diets supplemented with CLA at levels ranging from 0.05 to 0.5% by weight, as little as 0.1% CLA reduced mammary tumors, indicating that CLA is a potent anti-carcinogen.[31] Also, when CLA was given to rats during the pubertal period, a time of rapid morphological development of the mammary gland, the mammary tissue became less susceptible to cancer later in life.[33] This finding suggests that consuming an adequate intake of CLA early in life may have long-lasting beneficial effects on cancer risk.

Most studies have used mixed isomers of CLA. However, purified isomers of CLA, *c*9, *t*11 and *t*10, *c*12, as well as ruminant-derived CLA (i.e., a high CLA butter), which is predominantly the *c*9, *t*11 isomer, have also been shown to reduce mammary cancer in laboratory rats.[34] In addition, *c*9, *t*11 CLA derived endogenously from vaccenic acid by delta-9-desaturase has an anti-carcinogenic effect in rats.[35,36] In rats fed a diet enriched in vaccenic acid, CLA accumulated in the mammary fat pad and risk of mammary tumorigenesis decreased.[35,36]

Metastasis, or the spread of cancer, is the greatest cancer-related threat to life. Studies in experimental animals indicate that CLA (both *c*9, *t*11 and *t*10, *c*12 isomers and a mixture) reduces mammary cancer metastasis.[37,38] Moreover, new findings indicate that the type of dietary fat fed influences the effectiveness of dietary CLA in reducing mammary tumor metastasis.[39] In mice injected with mammary tumor cells and fed diets differing in the type of fat, metastasis was significantly reduced when beef tallow replaced half of a vegetable fat blend imitating the dietary fat composition of a typical American diet.[39] Beef tallow also increased the potency of CLA. Increasing beef tallow lowered the concentration of CLA needed to significantly reduce mammary tumor metastasis from 0.1% to 0.05% of the diet.[39] An in vitro study revealed that specific fatty acids in beef tallow (i.e., oleic, stearic, and palmitic acid) did not change or enhanced CLA's beneficial effects, whereas linoleic acid (the main fatty acid in vegetable oil) reduced the anti-carcinogenic effect of CLA on mammary tumor cells in cultures.[39] Another in vitro study showed that beef fatty acids (i.e., four fatty acid extracts prepared from beef lipid and varying in their CLA content) reduced the proliferation of human cancer cell lines (breast, colon, melanoma, and

ovarian) more than did their corresponding purified CLA-enriched fractions.[40] The researchers speculate that this finding may be explained by a complimentary effect of non-conjugated fatty acids and CLA in beef.

The mechanism(s) by which CLA reduces mammary (or other) cancer remains to be established.[8,26,29] However, findings suggest that CLA may reduce cell proliferation, inhibit eicosanoid formation, induce apoptosis (programmed cell death), inhibit angiogenesis (which would reduce the availability of nutrients to the tumor), regulate gene expression, and have antioxidative effects.[6,8,29] There is some evidence that the anti-carcinogenic effects of *c*9, *t*11 and *t*10, *c*12 CLA isomers may be mediated through different mechanisms.[6,8]

In contrast to *in vitro* and experimental animal studies, relatively few studies in humans have examined CLA's effects on mammary (and other) cancer. Moreover, the limited number of human studies that have been conducted are epidemiological (which do not prove a cause and effect relationship) and the findings are inconsistent.[6,8] Although an anti-carcinogenic effect of CLA in humans has yet to be established, researchers suggest that, based on the amount of CLA needed to reduce cancer in experimental animal studies, humans would need to substantially increase their CLA intake (i.e., from ~ 0.2 g/day to ~0.6 g/day) to elicit a cancer protective effect.[21]

Cardiovascular Disease

CLA has been shown to reduce cardiovascular disease risk factors such as atherosclerosis and blood lipids in experimental animals including rabbits, hamsters, and mice.[4,6,8,41-44] In rabbits, a mixture of CLA isomers (*c*9, *t*11 and *t*10, *c*12) and the two individual CLA isomers, *c*9, *t*11 and *t*10, *c*12, reduced the growth of atherosclerotic lesions to the same extent.[41] Similarly in hypercholesterolemic hamsters, *c*9, *t*11 and *t*10, *c*12 CLA isomers significantly lowered plasma total cholesterol and HDL cholesterol concentrations with no significant differences between these treatments.[42] However, the *c*9, *t*11 isomer lowered plasma triglyceride and glucose levels more than the *t*10, *c*12 isomer, suggesting a beneficial effect for *c*9, t11 CLA.[42] When researchers examined the effect of CLA (a 80:20 blend of *c*9, *t*11: *t*10, *c*12 CLA) on the regression of atherosclerosis in mice, they found that CLA not only prevented the progression, but also almost completely abolished atherosclerosis.[44] However, the effects of CLA on atherosclerotic lesions and blood lipid levels in experimental animal studies are conflicting.[8] Individual CLA isomers may exert different atherogenic effects.[45] A study in mice found that *c*9, *t*11 CLA impeded the development of atherosclerotic lesions, whereas *t*10, *c*12 CLA promoted atherosclerosis.[45]

Similar to studies in animal models, variations in findings have been reported in the few human investigations that have evaluated the effects of CLA (mixed or individual isomers) on atherogenic lipids and lipoproteins.[8,46] A 24-month study in healthy overweight adults found that a high dose of CLA (3.4 g/day) favorably affected blood lipid levels (i.e., reduced plasma total and LDL cholesterol with no change in HDL cholesterol and triglyceride levels).[47] Similar to findings in mice[45], opposing effects of *c*9, *t*11 CLA and *t*10, *c*12 CLA on blood lipid levels have been shown in healthy adults.[48] Specifically, *c*9, *t*11 CLA has a favorable effect on blood lipid levels, whereas *t*10, *c*12 CLA appears to have an unfavorable effect.[46,48] Before CLA or purified CLA isomers can be recommended for improving cardiovascular health in humans, more long-term studies in different populations are recommended.[8]

Body composition

CLA's effect on body composition was first demonstrated a decade ago in mice.[49] This study demonstrated that intake of CLA (0.5% by weight) decreased body fat mass and increased lean body mass. Subsequent research in several different animal models using CLA containing equal proportions of *c*9, *t*11 and *t*10, *c*12 isomers has confirmed and extended these findings.[6,8,50,51] The most dramatic results have been shown in mice as these animals appear to be particularly sensitive to CLA in losing fat mass. Studies using purified isomers or CLA enriched in either *c*9, *t*11 or *t*10, *c*12 isomers indicate that the *t*10, *c*12 is the primary isomer involved in reduction of fat mass.[8]

Relatively few studies have examined the effects of CLA or its isomers on body composition in humans. Results of these studies are less dramatic and more inconsistent than in experimental animals.[8,46] Nevertheless, some short- and long-term studies using high doses of CLA in healthy and obese, sedentary and exercised adults have shown beneficial effects of CLA in reducing fat mass and increasing lean body mass.[8,47,52] When healthy overweight and obese adults were supplemented with 3.4 g/day of CLA (mixed isomer) or a placebo for six months, body fat mass significantly decreased in specific regions (e.g., legs, abdomen) and lean body mass was maintained or increased compared to the placebo group.[52] Likewise, in a clinical trial of 40 healthy overweight adults, intake of 3.2 g CLA/day for six months significantly reduced body fat and helped prevent weight gain during the holiday season.[53] In contrast, other studies in adults have found at most a modest[54] or no[21] effect of CLA on body fatness. Clearly, more studies, particularly human trials in large groups of subjects, are needed before CLA can be recommended to improve body composition.[8,50]

Other Effects

Potential beneficial effects of CLA on insulin resistance, the metabolic syndrome, immune response, and bone health have been investigated.[6,8] The impact of CLA on insulin sensitivity is inconsistent.[6,8] In experimental animals, factors such as the duration of the study, metabolic state (normal vs. diabetic), and strain (mice vs. rats) and the CLA isomer(s) used influence the findings.[8] For example, feeding *t*10, *c*12 CLA to mice has been shown to induce adipose tissue inflammation and insulin resistance.[55] In contrast, feeding mice *c*9, *t*11 CLA has been demonstrated to improve insulin resistance and reduce hyperglycemia.[56] The effect of *c*9, *t*11 CLA on improving insulin sensitivity and management of diabetes was attributed to its anti-inflammatory effect.[56] Likewise in human clinical trials, the effects of CLA on insulin sensitivity appear to be isomer specific with *c*9, *t*11 CLA improving insulin sensitivity and *t*10, *c*12 CLA isomer showing a detrimental effect on insulin sensitivity.[6,8,57,58] Insulin resistance is the hallmark of the metabolic syndrome. The potential benefits of CLA on the metabolic syndrome remain controversial.[57,59,60]

CLA may modify mediators of immunity such as eicosanoids, prostaglandins, cytokines, and immunoglobulins.[8,61-63] *In vitro* and in vivo studies in various animal models demonstrate that CLA influences cytokine and prostaglandin production, which could influence the inflammatory response.[8] In experimental animals, CLA has been shown to modulate the immune system and prevent immune-induced wasting[62], as well as cancer-induced wasting.[64] Some studies in humans suggest a potentially beneficial effect of CLA on immune function.[61,63] A randomized controlled trial in 28 healthy adults showed that 3 g/day of CLA (mixed isomer) fed for 12 weeks improved cell-mediated immunity which allows the body to resist viruses, bacteria, fungi, and tumors.[63] Improvements in other markers of immune function with CLA supplementation indicated that CLA was capable of reducing inflammatory responses as well as allergic reactions.[63] However, the researchers call for further studies to confirm these findings and determine specific concentrations of individual isomers and mixtures of CLA to improve immune function.[63]

In vitro, experimental animal, and a few clinical trials support a beneficial effect of CLA on bone health.[8,65,66] In mice, CLA has been shown to increase bone mass at various sites by both reducing bone resorption and increasing bone formation.[65] In postmenopausal women, an average intake of 0.63 g of dietary CLA/day was associated with increased hip and forearm bone mineral density.[66] In contrast, a clinical trial in healthy adult males found that a CLA supplement of 3 g/day for 8 weeks had no effect on biomarkers of calcium and bone metabolism.[67]

Dietary Beef as a Source of CLA

Although most studies have used synthetic mixtures of CLA or individual isomers, there are several reasons why food sources of CLA, such as beef, may be preferable. Beef is a rich natural source of CLA, over 70% of which is the biologically active *c*9, *t*11 isomer shown to have anti-carcinogenic and anti-atherogenic effects as well as other possible health benefits.[9] Concern related to the undesirable health effects of *t*10, *c*12 CLA supplements[6,8,44] supports the intake of CLA from food sources. CLA obtained from ruminant-derived foods such as beef is relatively high in the *c*9, *t*11 isomer, with low levels of the *t*10, *c*12 isomer. Beef is a source of vaccenic acid (a "good" *trans* fat) which can be converted in the body to *c*9, *t*11 CLA.[24] Other components such as specific fats in beef may increase the effectiveness of CLA in reducing disease risk.[39] Although the minimum effective intake of CLA for disease prevention and overall health is unknown, beef provides more than 30% of current intake.[21] Studies indicate that the health benefits of CLA are achieved at intakes much higher than currently consumed.[8] One strategy to increase CLA intake is to raise the *c*9, *t*11 CLA content of beef fat by manipulating the diet of beef cattle and altering management practices on the farm, although the effects have been varied.[9,20] Importantly, beef is a naturally nutrient-rich food containing not only CLA, but many other nutrients (e.g., protein, zinc, vitamin B_{12}, etc) considered to be beneficial to health. Although much remains to be learned regarding the health benefits of CLA, foods naturally rich in CLA such as beef are being viewed as functional foods that provide health benefits beyond their basic nutrition. The American Dietetic Association, in a position statement on functional foods, recognizes beef (as well as lamb, turkey, and dairy foods) as a functional food due to its CLA content.[68]

Summary

In vitro and experimental animal studies indicate potential health benefits of CLA. The predominant CLA isomer in beef, *c*9, *t*11 (rumenic acid), has been demonstrated to inhibit cancer at several sites, particularly the mammary gland, reduce cardiovascular disease risk factors, improve insulin sensitivity, and exhibit an anti-inflammatory effect. However, relatively few studies have been conducted in humans. Moreover, there is considerable variation between and among findings from experimental animal and human studies investigating potential health benefits of CLA, which may be attributed to differences in the sources and amounts of CLA used, among other factors.

Findings to date warrant further investigation, particularly in humans, to substantiate CLA's health benefits and safety, determine the relative potency of natural sources of *c*9,

*t*11 CLA and its precursor, vaccenic acid, in foods such as beef, and to identify the minimum amount of CLA to confer health benefits. The need for further research on the health benefits of CLA in humans is recognized in the 2005 Dietary Guidelines Advisory Committee Report.[69] This report acknowledges the unique biological effects and potential importance of naturally occurring fatty acids, such as CLA and its precursor, vaccenic acid.

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