CONJUGATED LINOLEIC ACID:
A POTENT ANTICARCINOGEN FOUND IN MILK FAT

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Introduction

As an important source of nutrients, milk provides high quality protein, energy, calcium, and a variety of vitamins and minerals. Recent research has focused on altering the fat and protein content of milk and other dairy products in order to improve the nutrient content of these foods so that they more aptly reflect current dietary recommendations and trends. Conjugated linoleic acids (CLA), a component of milk fat, brings an exciting twist to the concept of redesigning foods as milk high in CLA may possess even greater human health benefits: anticarcinogenic capabilities.

Diet is a contributing factor to the onset or progression of some cancers. Epidemiological studies indicate diet composition may be related to 35 percent of human cancer deaths (1). A few substances in our diet have been identified as anti-carcinogens, but most are of plant origin and only present at trace concentrations. However, CLA are unique among naturally occurring anticarcinogens in that they are potent at extremely low levels and present in foods from ruminant animals.

Anticarcinogenic Effects of CLA

The National Academy of Science publication, Carcinogens and Anticarcinogens in the Human Diet, concluded ". . . conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals" (2). The term conjugated linoleic acid refers to a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Linoleic acid is an 18-carbon fatty acid; theoretically, CLA can have double bonds at several pairs of carbons including carbon positions 9 and 11, 10 and 12, or 11 and 13 and these can be in either the cis or trans configuration. The biologically active form of CLA for anticarcinogenic effects is thought to be the cis-9, trans-11 isomer. A slight bond shift is the difference between CLA and linoleic acid as illustrated in Figure 1. Linoleic acid at high dietary levels enhances carcinogenesis in laboratory animal studies whereas CLA inhibits carcinogenesis even at extremely low dietary concentrations (1,3). Therefore, the position of the double bonds must play a key role in CLA's anti-cancer capabilities.

Unlike most anticarcinogenic compounds, CLA can both reduce the incidence of new tumor formation in animal models and serve as a cytotoxic agent to existing tumor cells. Experimental models of mouse skin carcinogenesis, mouse forestomach tumorigenesis, and rat mammary tumorigenesis have been used to demonstrate CLA's ability to reduce the incidence of tumors. The cytotoxic effects of CLA have been shown in *in vitro* studies with melanoma, colon carcinoma, prostate carcinoma, leukemia, ovarian carcinoma, and breast tumors. An example of the effect CLA on tumor incidence is illustrated in Figure 2 (3). This study demonstrated that dietary CLA concentrations between 0.05 and 0.25% produced a dose dependent reduction in mammary tumor incidence when fed chronically to rats treated with a chemical carcinogen (Figure 2).

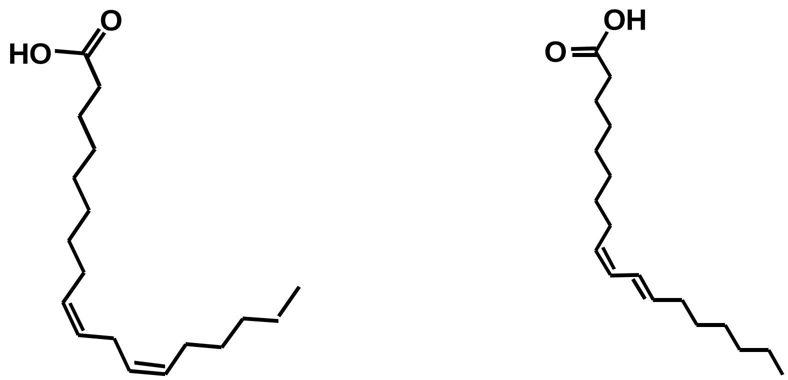

Figure 1. Chemical structure of linoleic acid (cis-9, cis-12 $C_{18:2}$) (left) and conjugated linoleic acid (cis-9, trans-11 $C_{18:2}$) (right). Note the shift in double bonds.

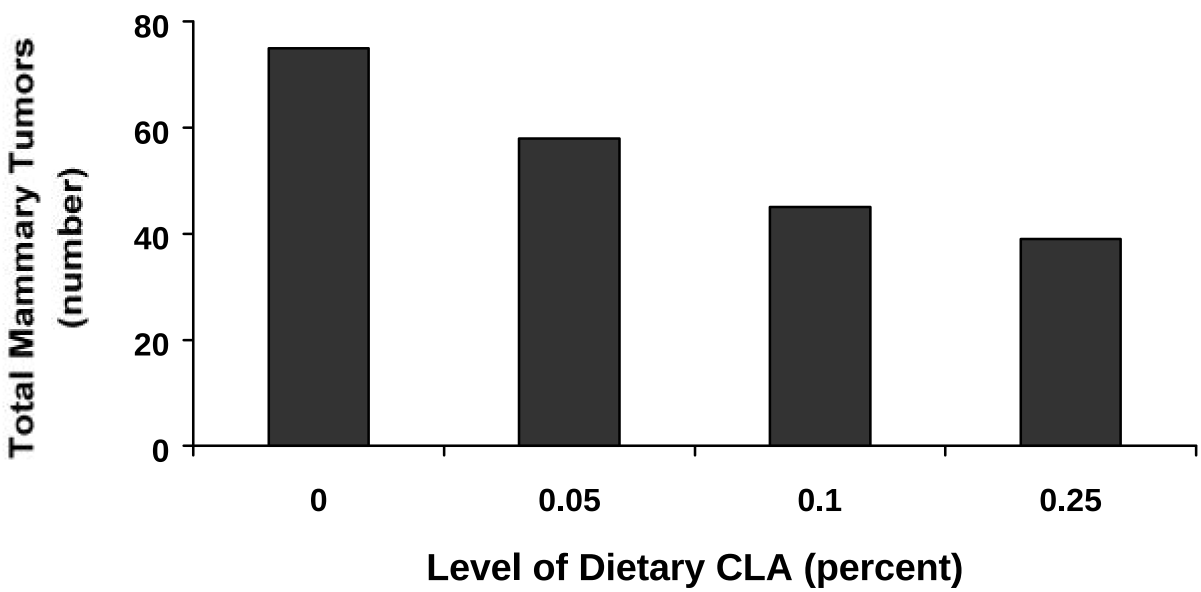

Figure 2. Effect of dietary CLA on the incidence of mammary tumors in rats. The model involves treating rats with the chemical DMBA to induce mammary tumor formation.

Dietary Sources of CLA

CLA is present in products from ruminant animals including milk, dairy products, and meat. Among these, dairy products are recognized as major dietary sources of CLA. Most of the CLA in milk and dairy products is the cis-9, trans-11 isomer, the form thought to be biologically active as an anticarcinogen. CLA concentration in foods are generally expressed as milligrams of CLA per gram of fat. Most dairy products range from 3 to 7 mg CLA/g of fat (Table 1), with concentrations of 4 to 5 mg considered average for homogenized milk (4). Furthermore, the CLA content is relatively stable over a range of processing, manufacturing, and storage conditions. Therefore, the CLA concentration in dairy products is essentially a function of the concentration in raw milk.

Table 1. CLA concentrations in common dairy products.

Foodstuff	CLA(mg/g fat)	
	Average	SEM
Homogenized milk	5.5	0.30
Condensed milk	7.0	0.29
Butter fat	6.1	0.21
Ice cream	3.6	0.10
Colby cheese	6.1	0.14
Mozzarella cheese	4.9	0.20
Plain yogurt	4.8	0.26

Currently, it is estimated that the average American consumes almost one-third of the CLA needed per day to achieve the intake which afforded cancer protection in animal models. Studies with animal models to examine the anticarcinogenic effects of CLA have demonstrated that diet is an effective route to provide cancer protection. However, all studies have added a purified form of CLA to the diet and none have used foods which have naturally high content of CLA. In addition, no epidemiological studies to date have examined the relationship between dietary CLA intake and risk of various cancers in humans. However, a recent Finnish National Public Health study compared the intake of dairy products and the risk of breast cancer. Results covered a 25-year period and indicated that as the intake of dairy products increased, the risk of breast cancer decreased. They concluded that there was a “protective effect” associated with consumption of milk that “overwhelms the associations between different other factors and risk of breast cancer” (5).

Additional positive health effects for CLA include antiatherogenesis, antiobesity and altered nutrient partitioning, enhancement of the immune system and the ability to prevent diabetes (4,6). These effects have all been demonstrated in animal models, and most studies have used a commercial CLA preparation that includes a mixture of CLA isomers. Thus, the specific isomer(s) which is responsible for each effect is unknown. We are currently collaborating with D. Barbano (Food Science, Cornell University) and C. Ip (Roswell Park Cancer Research Institute, Buffalo, NY) to examine the effect of a butter with a high concentration *cis*-9, *trans*-11 CLA on the incidence of tumors in a rat mammary-development model.

The Cow Dimension

CLA is found predominately in products of ruminant animals and is formed as a result of incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen (4). Linoleic acid is one of the major unsaturated fatty acids found in the diets of animals. The pathway by which it is biohydrogenated to stearic acid is presented in Figure 3. In the first step the double bond is moved over one position to form a conjugated fatty acid or CLA. To complete the biohydrogenation CLA goes through two additional steps involving successive additions of hydrogen to the double bonds resulting in the saturated fatty acid stearate. When biohydrogenation is not complete, CLA can escape the rumen and be absorbed from the digestive tract, thereby providing the mammary gland with a source of CLA which is found in milk fat.

The key to altering CLA production is to identify rumen conditions that favor stopping the biohydrogenation sequence with CLA as the end product over conditions that convert the CLA to complete the biohydrogenation process. The rumen microorganisms responsible for the biohydrogenation have been investigated (4). It turns out that rumen bacteria are primarily responsible and the rumen protozoa play little or no role. A range of diverse bacteria have been isolated and shown to have the capacity to biohydrogenate unsaturated fatty acids. No one species carries out the full sequence of reactions shown in Figure 3. Rather, it requires different rumen bacteria species to complete the conversion of unsaturated fatty acid to saturated fatty acids.

CLA concentrations in milk fat can vary greatly with different nutrition and management programs. A survey of milk from Canadian creameries demonstrated an eightfold variation in CLA levels. A similar tenfold variation was observed in a survey of New York dairy herds with a range of less than 2 to more than 15 mg CLA/g of milk fat. However, no relationship was observed between milk fat test and CLA levels in these herds. Thus, it is clear that current dietary and management practices used by dairy producers are resulting in a rumen environment in which the biohydrogenation of fatty acids varies so that milk fat concentrations of CLA also varies.

Linoleic Acid

(cis-9, cis-12 $C_{18:2}$)

↓

CLA (Conjugated Linoleic Acid)
(cis-9, trans-11 $C_{18:2}$)
↓
Trans Fatty Acid
(trans-11 $C_{18:1}$)
↓
Stearic Acid
(saturated $C_{18:0}$)

Figure 3. Pathway of biohydrogenation of linoleic acid to stearic acid by rumen microorganisms.

Over the last few years, several research groups have investigated the effect of various dietary and management factors on the milk fat content of CLA. We have collaborated with others in more than a dozen studies and found that many dietary factors can influence milk content of CLA. Table 2 represents a general summary of many of the factors we have identified. Most of these dietary factors result in a two to threefold increase in CLA as contrasted with the eight to tenfold differences observed between herds. Therefore, it seems likely that synergisms exist and additional factors may influence the rumen environment and microbial population. We have also observed substantial variation in CLA concentration in milk fat among cows at a similar stage of lactation, given a similar diet, and management regime. The trend appears to be that dietary factors which elicit the greatest increases in CLA concentrations in milk fat, also result in the greatest individual variation.

Results from our studies on dietary effects have also raised the possibility that rumen production may not be the only source of milk CLA. We hypothesized that the cow herself could synthesize CLA in the mammary gland from trans fatty acids with an enzyme called delta-9 desaturase. To test this we abomasally infused trans-11 $C_{18:1}$ fatty acids and indeed observed a substantial increase in milk fat content of CLA. Note that the trans-11 $C_{18:1}$ fatty acids are also an intermediate in the rumen biohydrogenation of dietary unsaturated fatty acids (Figure 1). Thus, in examining the dietary factors affecting milk fat content of CLA we need to focus on rumen production of both CLA and trans fatty acids.

Table 2. Listing of some factors which affect CLA concentrations in milk fat.

Dietary Factors	Effect on CLA content of milk fat

Rumen Environment	
Pasture vs TMR	Increased with pasture
Forage:concentrate ratio	Increased with high forage:concentrate ratio
Growth stage of forage	Increased with less mature forage
Dietary buffers	Little effect
Rumensin	Little effect
Nonstructural carbohydrate level	Minor effect
Intake level	Increased by underfeeding
Lipid Substrate	
Unsaturated fat vs saturated fat	Increased by addition of unsaturated fat
Level of plant oils	Increased by higher levels
Plant oil type	Greatest with oils high in $C_{18:2}$
Ca salts of plant oils	Increased as with free oils
Animal fat byproducts	Minimal effect
High oil corn (grain and silage)	Minimal increase
Fish oils	Increased in relation to level
Processing of soybeans	Increased over raw beans

Summary

CLA are unique among naturally occurring anticarcinogenic substances in that it is potent at extremely low levels and is present in foods from ruminant animals. CLA are produced in the rumen as an intermediate in the biohydrogenation of unsaturated fatty acids by rumen bacteria. In turn CLA are absorbed from the digestive tract and incorporated into milk fat. CLA can also be formed by the cow herself by an enzymatic conversion of trans-11 fatty acid, an another biohydrogenation intermediate formed in the rumen. As a result, dairy products are the major source of CLA in the human diet. Analysis of milk from different herds indicates substantial natural variation exists and that CLA content in milk fat of dairy cows can be markedly increased via dietary manipulation. Many dietary factors influence CLA production in the rumen and result in altered CLA concentrations in milk fat. Enriching foods with naturally occurring anticarcinogens, such as CLA, has the potential to greatly improve the nutritional quality of milk and milk products for the benefit of all who consume them.

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