

Conjugated Linoleic Acid (CLA) and Milk Fat: A Good News Story

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ABSTRACT

Milk fat contains several compounds with known anti-carcinogenic properties. One of these is conjugated linoleic acid (CLA), and dairy products are the major source of CLA in human diets. In addition to its anti-cancer effects, biomedical studies with animal models have demonstrated that CLA has a range of beneficial effects. The processes for the biosynthesis of CLA in dairy cows have been established and involve the absorption of fatty acid biohydrogenation intermediates produced in the rumen and their subsequent conversion to CLA by the mammary enzyme Δ^9 -desaturase. Studies have further identified the substantial effect of dietary factors on milk fat content of CLA and identified a marked range among individual cows in the ability to synthesize CLA. Thus, through diet manipulation and perhaps even genetic selection, the milk fat content of CLA can be enhanced. Overall, the presence of CLA in milk fat is a clear example that milk contains microcomponents that provide benefits beyond those associated with traditional nutrients.

INTRODUCTION

Milk and dairy products are important dietary sources of nutrients, providing energy, high quality protein, 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 their nutrient content to more aptly reflect current dietary recommendations and trends. However, there is increasing recognition that foods can also be contributing factors in the prevention and development of some disease conditions. For example, it has been estimated that approximately one-third of cancer deaths may be related to diet (Parodi, 1999). As a result, additional focus is being given to designing foods that have beneficial effects on human health.

The term “functional foods” is often used as a generic description for the beneficial effects of ingested foods that go beyond their traditional nutritive value (Bauman et al., 2001). Parodi (1997; 1999) has written a number of reviews summarizing the role of dairy products in the prevention of cancer. As listed in Table 1, a number of milk fat components have been identified as having anti-carcinogenic properties, and one that has received recent focus is conjugated linoleic acid (CLA). The discovery of a “functional food” role for CLA occurred over two decades ago when Pariza and coworkers found that ground beef contained an anti-mutagenic factor that consisted of a series of conjugated dienoic isomers of linoleic acid (Pariza et al., 2001). Subsequent work demonstrated that chemically synthesized CLA was able to reduce the incidence of several types of tumors in animal models. Most naturally occurring anti-carcinogens are present only at trace levels and of plant origin. However, CLA is unique among naturally occurring anti-carcinogens in that it is potent at extremely low levels and present in dairy products and meat from ruminant animals.

As the biomedical studies with CLA expanded, it became apparent that CLA had a range of positive health effects in experimental animal models (Table 2). These included beneficial effects on reducing body fat accretion, delaying the onset of type II diabetes, retarding the development of atherosclerosis, improving the mineralization of bone and modulating the immune system (Belury, 2002). This has resulted in an exponential increase in CLA-related research over the last few years. The following sections will provide background and review the biology of CLA related to dairy production.

BACKGROUND

Conjugated linoleic acids represent a mixture of positional and geometric isomers of an 18 carbon fatty acid with two double bonds. The double bonds pairs in fatty acids generally have a methylene group between them, but in CLA they have a conjugated arrangement. The presence of fatty acids with conjugated double bonds was first demonstrated in food products by Booth et al. (1935) working with milk fat from cows turned out to spring pasture. Theoretically, a number of CLA isomers are possible that differ in the positions of the double bond pairs (e.g 7-9, 8-10, 9-11, 10-12, and so forth). The two forms (isomers) of CLA that have been most studied are *cis*-9, *trans*-11 and *trans*-10, *cis*-12. In Figure 1, their structures are contrasted with linoleic acid, an essential dietary fatty acid that also contains 18 carbons and 2 double bonds. Additional differences among CLA isomers can exist in the configuration of the double bond so that *cis-trans*, *trans-cis*, *cis-cis* or *trans-trans* configurations are all possible. Most studies with animal models have used CLA from commercial sources; industrial manufacture of CLA results in products that contain significant quantities of many CLA isomers, although this varies with the manufacturing process (Banni and Martin, 1998).

Parodi (1977) was the first to demonstrate the presence of *cis*-9, *trans*-11 CLA in milk fat. This is the major isomer and it represents about 75-90% of the total CLA in milk fat. This is of special importance because the *cis*-9, *trans*-11 CLA isomer has been shown to be anticarcinogenic in biomedical studies with animal models. Indeed, utilizing a breast cancer model, a recent study demonstrated that rats fed a diet containing butter that was naturally enriched with *cis*-9, *trans*-11 CLA had a lower mammary tumor incidence and fewer tumors than rats consuming the control diet (Ip et al. 1999). The second most prevalent CLA isomer in milk fat is *trans*-7, *cis*-9 and its concentration is about 10% of that for *cis*-9, *trans*-11. To date, the specific biological effects of *trans*-7, *cis*-9 CLA have not been investigated because of its limited availability. In addition, milk fat content of *trans*-10, *cis*-12 CLA can be markedly increased under certain dietary situations, but even in this instance the amount is less than 2% of the *cis*-9, *trans*-11 CLA content. The presentation by Dr. Baumgard demonstrated that the *trans*-10, *cis*-12 CLA isomer is a potent inhibitor of milk fat synthesis. Recently, investigations using the more elaborate analytical methods have revealed that milk fat contains trace concentrations of many additional isomers of CLA. For example, analysis of commercial cheese products demonstrated that *cis*-9, *trans*-11 was the predominant isomer (78-84%), but additional isomers of CLA were identified which resolved into seven *trans-trans* (5-9%), three *cis/trans* (*cis-trans* or *trans-cis*) (10-13%) and five *cis, cis* (<1%) isomers (Sehat et al., 1998).

BIOSYNTHESIS OF CLA

The uniqueness of CLA in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen. The lipid composition of forages consists largely of glycolipids and phospholipids, and the major fatty acids are two unsaturated fatty acids, linoleic acid (*cis*-9, *cis*-12 C_{18:2}) and linolenic acid (*cis*-9, *cis*-12, *cis*-15 C_{18:3}) acid. In contrast, the lipid composition of seed oils used in concentrate feedstuffs is predominantly triglycerides containing linoleic and oleic acid (*cis*-9 C_{18:1}) as the major fatty acids. When consumed by ruminant animals, dietary lipids undergo two important transformations in the rumen (Keeney, 1970). The initial transformation is hydrolysis of the ester linkages catalyzed by microbial lipases to release the fatty acids into the rumen fluid. This step is a prerequisite for the second transformation – biohydrogenation of the unsaturated fatty acids by rumen bacteria.

The biohydrogenation sequence of linoleic acid that occurs in the rumen is presented in Figure 2. Isomerization of the *cis*-12 double bond represents the initial step and this results in the formation of *cis*-9, *trans*-11 CLA. The second reaction is a reduction where *cis*-9, *trans*-11 CLA is converted to *trans*-11 C_{18:1} (also called vaccenic acid) and the final step is a second reduction resulting in the formation of stearic acid, a saturated fatty acid (C_{18:0}). The first two steps are rapid whereas the third reaction is slow. Therefore, vaccenic acid reduction tends to be rate limiting in the biohydrogenation sequence of unsaturated eighteen carbon fatty acids. As a consequence, this penultimate biohydrogenation intermediate accumulates in the rumen (Keeney, 1970; Harfoot and Hazlewood, 1997) and is, therefore, more available for absorption (Figure 2).

There is a close linear relationship between the milk fat content of vaccenic acid and CLA (Griinari and Bauman, 1999). Based on this and the fact that both of these are intermediates in the biohydrogenation by rumen bacteria, it been assumed that rumen production was the source of the CLA found in milk fat. However, a close relationship between vaccenic acid and *cis*-9, *trans*-11 CLA in milk fat would also be consistent with a precursor-

product relationship. Based on this and the kinetics of rumen biohydrogenation which would lead to vaccenic acid being available for absorption, Griinari and Bauman (1999) proposed that a portion of the CLA in ruminant fat was of endogenous origin. They hypothesized that endogenous *cis*-9, *trans*-11 CLA would originate in the mammary gland from the desaturation of vaccenic acid by Δ^9 -desaturase, and examined this in a series of studies. Results demonstrated that endogenous synthesis via Δ^9 -desaturase represented the predominant source of *cis*-9, *trans*-11 CLA in milk fat over a wide range of diets (Griinari et al., 2000; Corl et al., 2001; Lock and Garnsworthy, 2002; Piperova et al., 2002). In the case of pasture based diets, endogenous synthesis of *cis*-9, *trans*-11 CLA in the mammary gland represented over 87% of the total milk fat content of this CLA isomer (Kay et al., 2002). In more recent studies endogenous synthesis via Δ^9 -desaturase in the mammary gland has also been shown to be the source of *trans*-7, *cis*-9 CLA in milk fat (Corl et al., 2002; Piperova et al., 2002). In contrast, other CLA isomers found in trace amounts in milk fat appear to originate solely as intermediates from incomplete biohydrogenation in the rumen.

FACTORS AFFECTING MILK FAT CONTENT OF CLA

A number of investigations have examined the effect of manufacturing and storage practices on the concentration of CLA in dairy products. In general, results demonstrate that processing and storage have minimal effects indicating that CLA are relatively stable (Shantha et al., 1995; Banni and Martin, 1998). Thus, the content of CLA in dairy products is, in large part, dependent on the concentration of CLA in raw milk fat. The CLA are fatty acids so their concentrations in foods are generally expressed in relation to total fat. Typically dairy products have concentrations in the range of 3 to 7 mg/g of fat, but the concentration in milk fat can vary widely and may be several fold greater. For example, an 8-fold range in CLA content of milk fat was observed in surveys of Canadian creameries (Riel, 1963) and dairy herds in the Northeast U.S. (Kelly and Bauman, 1996). Overall, the content of CLA in fat from ruminant-derived food products will be dependent on the ruminal production of vaccenic acid and to a lesser extent CLA, and the tissue activity of Δ^9 -desaturase. Differences among herds are largely related to diet. However, even under identical diet and management conditions, there is still substantial variation among cows in the milk fat content of CLA.

Dietary Factors

The substantial variation in content of CLA in milk fat between herds suggests that diet has a major influence. Over the last 5 years, research has identified many dietary factors that affect the CLA content of milk fat. Table 3 presents an overview where dietary effects are grouped into categories relative to the potential mechanism by which they may act (Griinari and Bauman, 1999; Bauman et al., 2001). The first category includes dietary factors that provide lipid substrate for rumen biohydrogenation. Plant oils high in linoleic and linolenic acids are particularly effective. These lead to increases in rumen outflow of vaccenic acid and to a lesser extent, CLA isomers. The second grouping consists of dietary factors that alter the rumen environment thereby affecting the bacteria involved in rumen biohydrogenation. Forage to concentrate ratio plays an important part and can markedly affect the rumen environment and biohydrogenation. The third group includes dietary factors that involve a combination of lipid substrate and modification of the rumen population of bacteria. Pasture is one example and typically cows on lush spring pasture will have a milk fat content of CLA that is 2- to 3-fold greater than corn-based total mixed rations. However, as pasture matures, this difference in CLA diminishes.

The milk fat and body fat content of CLA can also be increased by adding dietary supplements of CLA (Table 3). Studies with dairy cows have generally involved abomasal infusion of the supplement as an experimental means to by-pass rumen fermentation processes. However, technologies exist to protect supplements from alterations by rumen bacteria (Doreau et al. 1997), and these have been successfully used to produce the fat supplements that are commercially used for lactating dairy cows. Investigations have established that dietary supplements of CLA result in dose-related increases in the concentration of CLA in milk fat. Supplements have contained a number of CLA isomers, primarily *trans*-8, *cis*-10 CLA, *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and *cis*-11, *trans*-13 CLA, and results demonstrated that all isomers of CLA were transferred to milk fat (Chouinard et al., 1999a; 1999b; Baumgard et al. 2000).

Animal Variation

Animal variation is also a major source of differences in the milk fat content of CLA. Studies involving diets ranging from total mixed rations to pasture have reported a several-fold range in milk fat content of CLA for

individual cows (Jiang et al., 1996; Kelly et al., 1998a; 1998b; Lawless et al., 1998; White et al., 2001). We have examined this in more detail and found that the hierarchy (rank order) for milk fat content of CLA is consistent over time among individuals and maintained even when cows are switched among diets that result in different overall averages in the milk fat content of CLA (Peterson et al., 2002; Kelsey et al., 2002).

The range among individuals in milk fat CLA is shown in Figure 3 (Kelsey et al., 2002), and the basis for these individual differences appears to be two-fold. First, cows appear to differ in ruminal production of vaccenic acid and CLA, even when all animals are consuming the same diet. This variation is presumably related to individual differences in factors such as feeding pattern and chewing frequency that would affect the rumen environment. Second, cows differ in Δ^9 -desaturase activity and endogenous synthesis of CLA in the mammary gland. Presumably, this variation among individuals would reflect differences in Δ^9 -desaturase involving regulation of gene expression, gene polymorphisms that affect the primary or tertiary structure of the enzyme, post-translational modifications, or factors that would affect the interaction between enzyme and the substrate or product (Peterson et al., 2002). This clearly offers potential for genetic selection and is an obvious area for future research.

IMPLICATIONS

Food products from ruminants contain conjugated linoleic acids (CLA), fatty acids that have beneficial health effects as shown in research with animal models. The biosynthesis of CLA and dietary factors that cause variation in the content of CLA in milk fat have been identified. Overall, the opportunity exists to substantially increase the concentration of CLA in dairy products. There are many isomers of CLA in milk fat but *cis*-9, *trans*-11 CLA predominates. Research with animal models has demonstrated this specific isomer has anticarcinogenic properties and mammary tumors are reduced when a CLA-enriched butter is fed. Thus, consideration of functional foods containing CLA represents an exciting research area of potential importance in the production of dairy products.

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Table 1. A partial list of milk fat components shown to have anti-carcinogenic properties.

Conjugated linoleic acids
Butyric acid
Vaccenic acid
Ether lipids
Sphingomyelin
Vitamin A and carotene

Adapted from Parodi 1997; 1999.

Table 2. Partial List of beneficial health effects of CLA reported from biomedical studies with animal models.

Biological Effect
Anticarcinogenic (in vivo and in vitro studies)
Antiatherogenic
Altered nutrient partitioning and lipid metabolism
Antidiabetic (type II diabetes)
Immunity enhancement
Improved bone mineralization

Adapted from Bauman et al. (2001).

Table 3. Overview of dietary factors that affect milk fat content of CLA.

Dietary Factor	Milk Fat Content of CLA
Lipid substrate	
Polyunsaturated fatty acids	increased
Saturated fatty acids	no effect
Modification of rumen environment	
Forage:concentrate ratio	increased as forage increases
Fish oils	increased
Buffers	minimal effect
Combination	
Pasture	increased
Forage maturity	decreased as maturity increases
CLA supplements	increased

Adapted from Griinari and Bauman (1999) and Bauman et al. (2001).

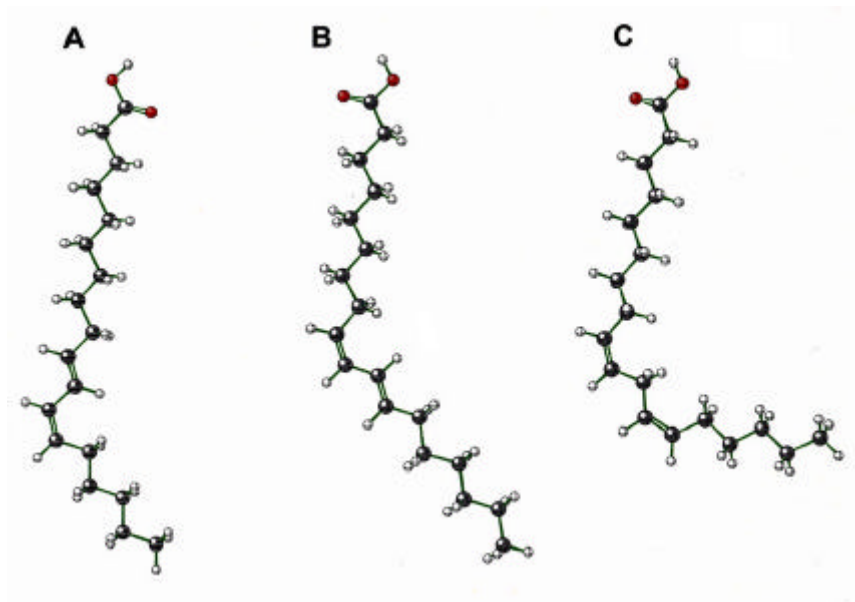


Figure 1. Chemical structure of conjugated linoleic acid isomers (CLA) and linoleic acid. Fatty acids are *trans*-10, *cis*-12 CLA (A), *cis*-9, *trans*-11 CLA (B) and *cis*-9, *cis*-12 octadecadienoic acid (linoleic acid) (C). Adapted from Bauman et al. (2000).

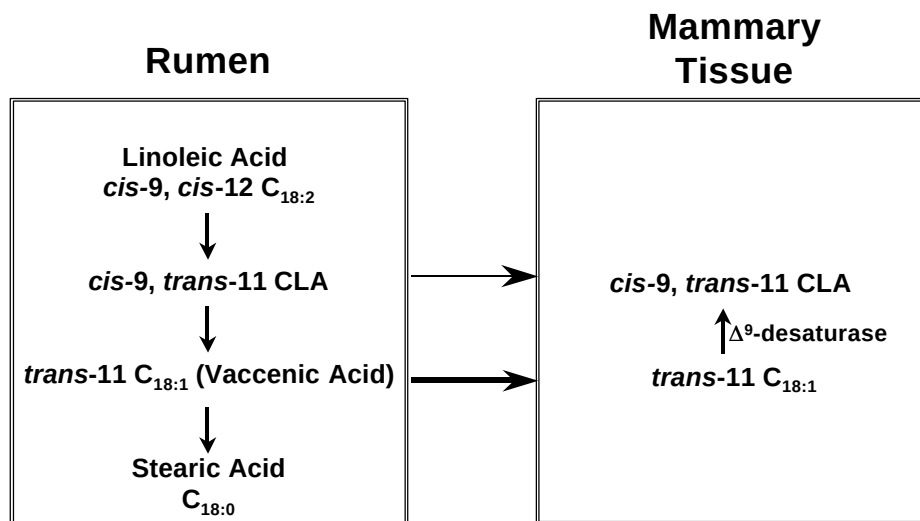


Figure 2. Role of rumen biohydrogenation and tissue Δ^9 -desaturase in the production of *cis*-9, *trans*-11 conjugated linoleic acid in milk fat. Adapted from Bauman et al. (2000).

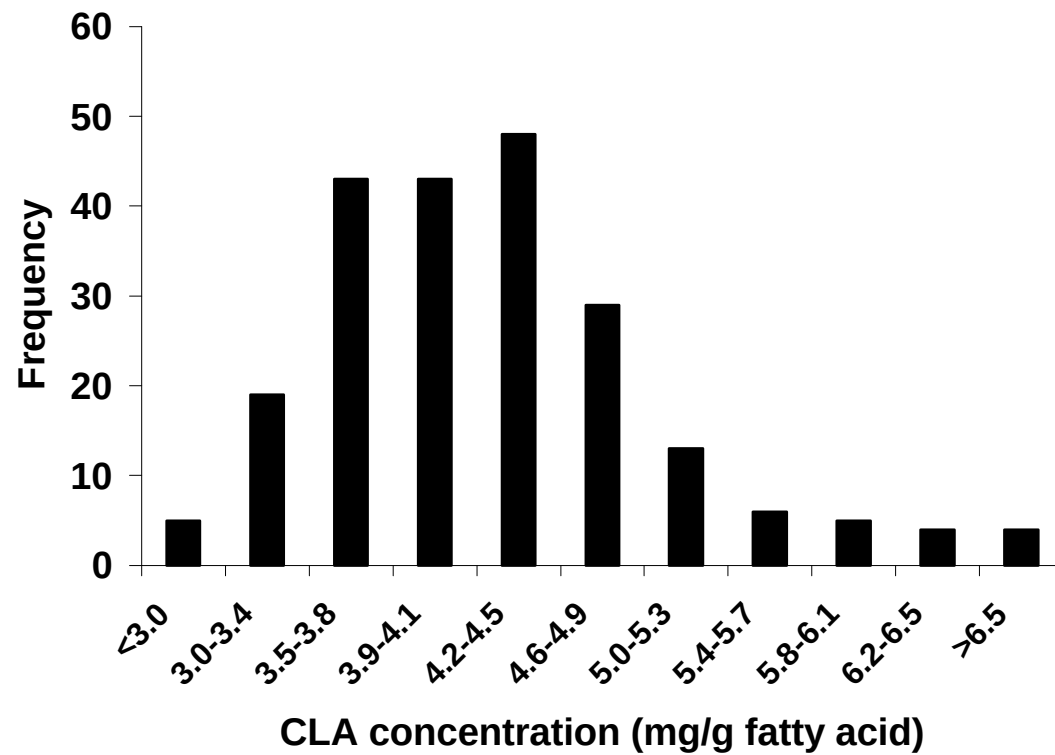


Figure 3. Distribution of milk content of CLA for 219 Holstein and Brown Swiss cows in the University of Arizona herd. All cows were fed the same diet. Adapted from Kelsey et al. (2002).