REVIEW ARTICLE

Occurrence of conjugated linoleic acid in ruminant products and its physiological functions

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ABSTRACT

Milk and meat products derived from ruminants contain a mixture of positional and geometric isomers of $C_{18:2}$ with conjugated double bonds, and *cis*-9, *trans*-11$C_{18:2}$ (conjugated linoleic acid, CLA) is the predominant isomer. The presence of CLA in ruminant products relates to the biohydrogenation of unsaturated fatty acids by rumen bacteria. Although, it has been suggested that *cis*-9, *trans*-11 CLA is an intermediate that escapes complete ruminal biohydrogenation of linoleic acid, is absorbed from the digestive tract, and transported to tissues via circulation. Its major source is endogenous biosynthesis involving Δ^9-desaturase with *trans*-11$C_{18:1}$ produced in the rumen as the substrate. CLA has recently been recognized in animal studies as a nutrient that exerts important physiological effects, including anticarcinogenic effects, prevention of cholesterol-induced atherosclerosis, enhancement of the immune response, reduction in fat accumulation in body, ability to enhance growth promotion, antidiabetic effects and improvement in bone mineralization. The present review focused on the origin of CLA in ruminant products, and the health benefits, metabolism and physiological functions of CLA.

KEYWORDS: conjugated linoleic acid, physiological functions, rumen hydrogenation, ruminant products, *trans*-11$C_{18:1}$.

INTRODUCTION

It is well known that food products derived from ruminants are high in saturated fatty acids (SFA) and have a lower polyunsaturated fatty acids (PUFA) : SFA ratio than non-ruminants because of the biohydrogenation of dietary unsaturated fatty acids in the rumen. In general, the consumption of SFA has been associated with an increased serum low-density lipoprotein (LDL) cholesterol level, which is a risk factor for coronary heart disease. Thus, ruminant products with a high level of SFA have been an easy target in the campaign for the strict control of fat intake. Consumer demand for food products of superior health quality has renewed interest in modifying the fatty acid composition of dairy milk. However, the fatty acid compositions of dairy milk and beef are highly resistant to manipulation because of ruminal biohydrogenation of dairy PUFA. Fortunately, it has been shown in recent years that food products derived from ruminants are the richest natural sources of conjugated linoleic acid (CLA), in particular *cis*-9, *trans*-11$C_{18:2}$, which is believed to have several important physiological functions, including anticarcinogenic, antiatherogenic, immunomodulating, growth promotion and lean body mass promotion. This is good news at last for the livestock industry. Thus, increasing the concentration of CLA in milk may be beneficial to public health and may enhance the consumption of dairy products.

In this paper, I will first describe the origin of CLA and describe how the CLA concentration in ruminant products can be enhanced. Second, I will review the physiological functions of CLA.

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Received 14 January 2005; accepted for publication 1 March 2005.

Origin of conjugated linoleic acid in ruminant products

Conjugated linoleic acid is a collective term describing a mixture of geometric and positional conjugated dienoic isomers derived from linoleic acid. In 1935, the presence of fatty acids with conjugated double bonds was first reported in milk fat from cows grazing on spring pasture (Booth *et al*. 1935), and subsequent work by Parodi (1977) demonstrated that it was primarily *cis*-9, *trans*-11 CLA. As analytical techniques improved, it became evident that ruminant fat contained many isomers of CLA (Sehat *et al*. 1998; Yurawecz *et al*. 1998). *Cis*-9, *trans*-11 CLA is the predominant isomer, representing 75–90% of the total CLA in ruminant fat, and *trans*-7, *cis*-9 CLA is the second most prevalent isomer at 3–16% of the total CLA. The common name "rumenic acid" has been proposed for the *cis*-9, *trans*-11 CLA isomer because of its unique relationship to ruminants (Kramer *et al*. 1998).

An extensive series of studies examined ruminal biohydrogenation of PUFA during the 1970s and 1980s (Keeney 1970; Tanaka 1974; Harfoot & Hazlewood 1988). The first transformation that occurs in the rumen is hydrolysis of the ester linkages by rumen microbial lipases to produce free fatty acids (Fig. 1). The second transformation is the biohydrogenation of PUFA. The major PUFA in the diets of ruminants are linoleic and α-linolenic acids. The predominant pathway for the biohydrogenation of these acids is shown in Fig. 2. *Cis*-9, *trans*-11 CLA is an intermediate in the biohydrogenation of linoleic acid to stearic acid. Parodi (1977) identified the major conjugated octadecadienoic fatty acid in milk fat as *cis*-9, *trans*-11 CLA. Furthermore, a close linear relationship has been observed between the milk fat content of *trans*-11$C_{18:1}$ (vaccenic acid) and *cis*-9, *trans*-11 CLA (Jahreis *et al*. 1997; Solomon *et al*. 2000). The intermediates that escape complete biohydrogenation in the rumen are absorbed from the digestive tract, and transported to

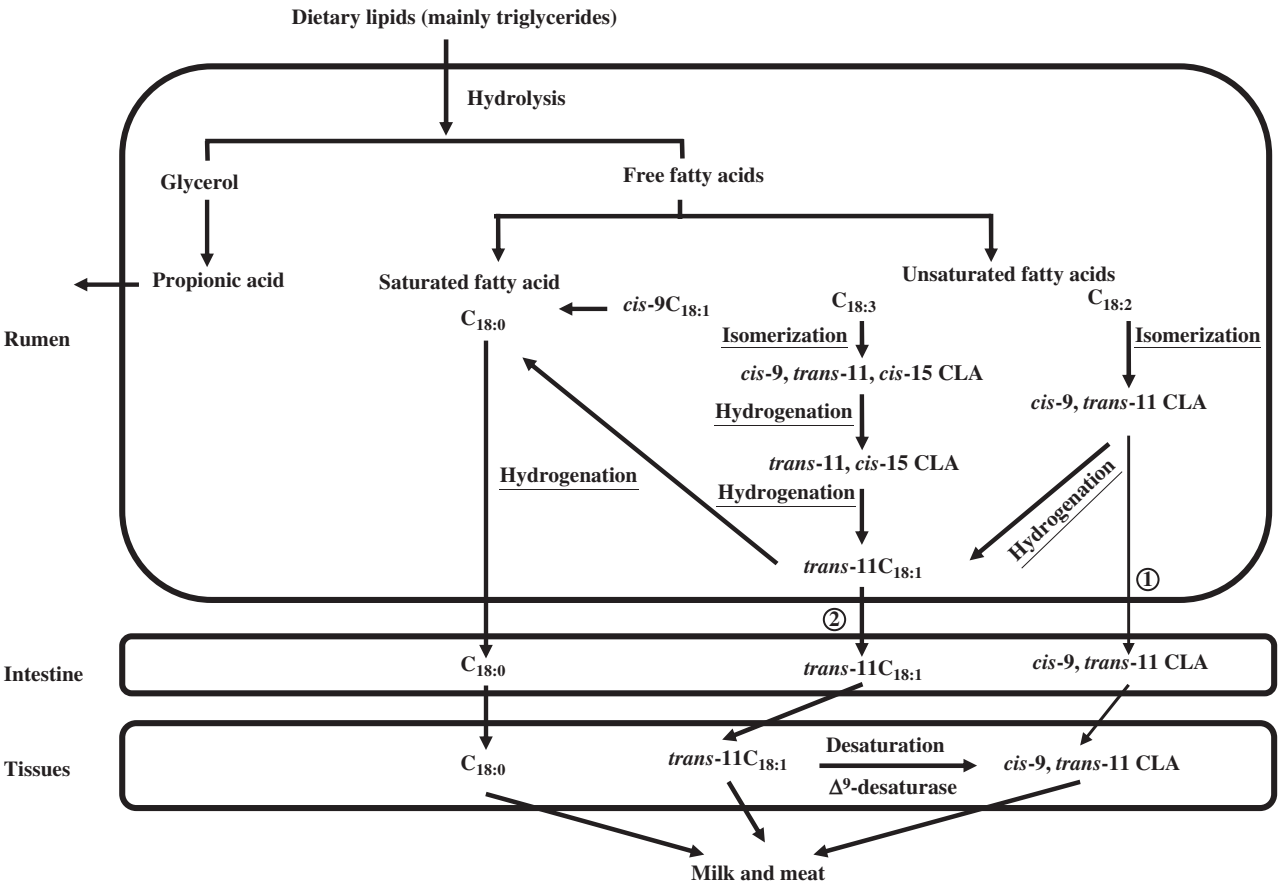

Fig. 1 Lipid metabolism in the rumen and the origins of conjugated linoleic acid in ruminant products.

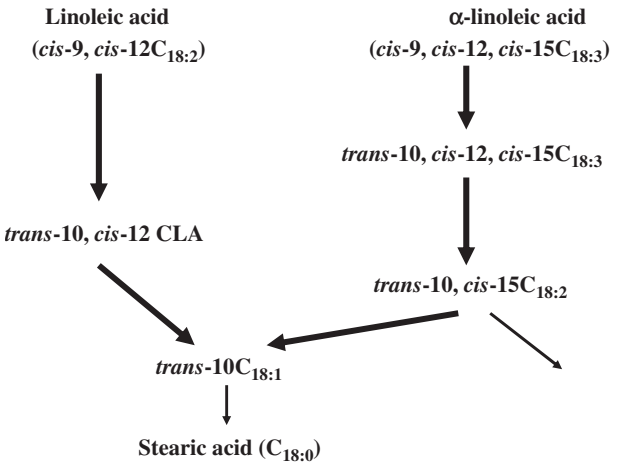

Fig. 2 Putative pathways of ruminal hydrogenation of linoleic and α-linoleic acid in cows fed a high-concentrate diet (adapted from Griinari and Bauman (1999)).

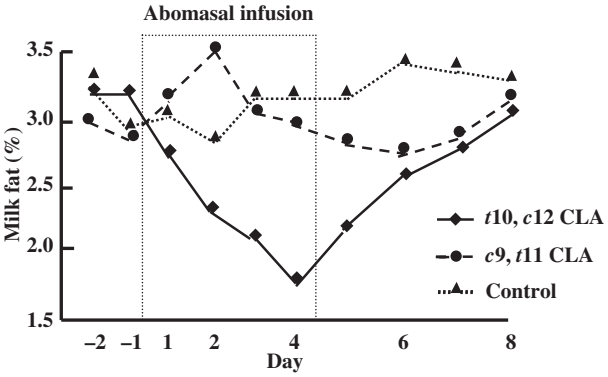

Fig. 3 Temporal pattern of milk fat percentage during abomasal infusion of conjugated linoleic acid isomers (adapted from Baumgard *et al*. (2000)).

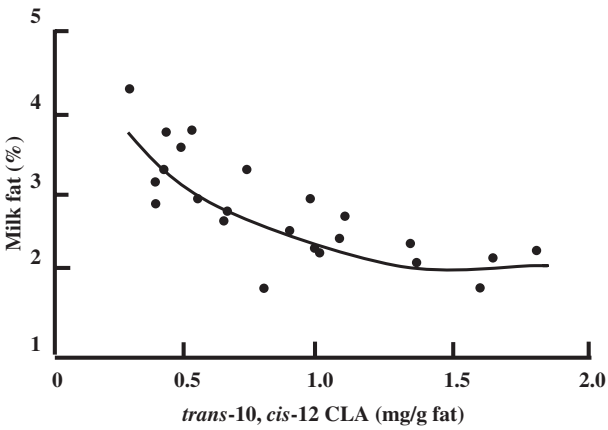

Fig. 4 Relationship between the milk fat content of trans-10, cis-12 conjugated linoleic acid (CLA) and the milk fat percentage of cows fed a low-fiber diet supplemented with sunflower oil (adapted from Griinari *et al*. (1999)).

the mammary gland via circulation. It was assumed that this was the origin of *cis*-9, *trans*-11 CLA in milk fat (route ① in Fig. 1). Alternatively, kinetic studies on Δ^9-desaturase derived from rat liver demonstrated that *trans*-11$C_{18:1}$ is converted to *cis*-9, *trans*-11 CLA by this enzyme, although the conversion of stearic acid to oleic acid represents the preferred substrate–product reaction (Mahfouz *et al*. 1980; Pollard *et al*. 1980). Furthermore, in a study by Griinari *et al*. (2000), the abomasal infusion of *trans*-11$C_{18:1}$ (12.5 g/day) into lactating dairy cows resulted in a 31% increase in the milk fat content of *cis*-9, *trans*-11 CLA. From these reports, it is assumed that endogenous synthesis of *cis*-9, *trans*-11 CLA also occurrs, and this involves the enzyme Δ^9-desaturase, with the precursor being *trans*-11$C_{18:1}$, another intermediate in the rumen biohydrogenation of linoleic and α-linolenic acids (route ② in Fig. 1).

Izumi *et al*. (2002) and An *et al*. (2003) investigated the occurrence and time-dependent changes of CLA and *trans*-11$C_{18:1}$ in the rumen using sheep fitted with a rumen fistula. Feeding sheep diets containing polyunsaturated fats results in a marked increase in the level of *trans*-11$C_{18:1}$ and a slight increase of CLA in the rumen (Figs 3, 4). In those studies, the level of *trans*-11$C_{18:1}$ (0.3–0.4 mg/g) was markedly higher than that of CLA (less than 0.05 mg/g). This suggests that the conversion rates of linoleic and α-linolenic acid into *trans*-11$C_{18:1}$ are more rapid than that of *trans*-11$C_{18:1}$ to stearic acid. Therefore, the *cis*-9, *trans*-11 CLA produced by the ruminal biohydrogenation of linoleic acid is transient intermediate, whereas *trans*-11$C_{18:1}$ is accumulated in the rumen. If the CLA in ruminant products originates from CLA that has escaped incomplete biohydrogenation of dietary linoleic acid in the rumen as generally recognized, the concentration of CLA in the rumen seems to be too low. Thus, the majority of *cis*-9, *trans*-11 CLA in milk fat seems to originate from endogenous synthesis via Δ^9-desaturase, and *trans*-11$C_{18:1}$ derived from rumen output is the precursor for endogenous synthesis (route ② in Fig. 1). This may explain why dietary supplements of plant oils high in α-linolenic acid produce an increase in milk fat CLA (Kelly *et al*. 1998a; Dhiman *et al*. 2000), even though *cis*-9, *trans*-11 CLA is not an intermediate in the ruminal biohydrogenation of α-linolenic acid as shown in Fig. 1. Thus, the key to increasing the milk fat content of *cis*-9, *trans*-11 CLA

should be to enhance ruminal output of *trans*-11$C_{18:1}$ and to increase the activity of Δ^9-desaturase in the mammary gland.

Influence of diets on the conjugated linoleic acid content of ruminant products

It is clear from many studies that altering the diet of ruminants, such as supplementing the diet with fats in the form of oilseeds or vegetable oils, can have a major effect on the CLA content of ruminant products. Pasture feeding results in a much higher milk fat content of CLA compared with feeding either a total mixed ration with a similar lipid content, or conserved forage (Jahreis *et al*. 1997). It has been found that the CLA content in the milk fat of pasture-fed cows is twofold (1.09 vs 0.46 g/100 g milk fat; Kelly *et al*. 1998b) to fivefold (2.21 vs 0.39 g/100 g milk fat; Dhiman *et al*. 1999) higher than that in cows given a total mixed ration. This is in accordance with the latest reports by Stockdale *et al*. (2003) and Schroeder *et al*. (2003), who observed an elevated CLA content in milk fat with pasture feeding. One reason why fresh grass promotes such increases is that the major fatty acids in fresh grass are linoleic and α-linolenic acids. α-Linolenic acids comprise more than 50%, whereas the preservation of grass, particularly ensilage, causes the loss of these fatty acids. In the silo, microorganisms live in an anaerobic environment similar to that in the rumen, which means that the disposal of hydrogen or hydrogen equivalents is a priority activity and, as in the rumen, unsaturated fatty acids are reduced. Izumi *et al*. (2002) reported that the percentage of α-linolenic acid decreases to half, and simultaneously, the total fatty acids decrease to one-third, from fresh grass to hay. Therefore, the amount of α-linolenic acid in hay is reduced to approximately one-sixth of that in fresh grass (Table 1). Furthermore, part of the increase may be a result of the increase in the activity of Δ^9-desaturase in the mammary glands of cows fed fresh grass. The feeding of fresh grass rather than conserved materials or concentrates provides more linoleic and α-linolenic acids as precursors of CLA, and appears to be a simple and effective means of enhancing the CLA content in ruminant products.

Dietary supplementation of plant oil or seeds with a high plant oil content results in substantial increases in the CLA content in milk fat. In a study by Kelly *et al*. (1998a), the CLA contents were 13.3, 24.4 and 16.7 mg/g milk fat, when cows were fed a supplemented diet with 53 g/kg dry matter of peanut oil (oleic acid, 51.5%), sunflower oil (linoleic acid, 69.4%), or linseed oil (α-linolenic acid, 51.4%), respectively. The increase in the CLA content observed with the sunflower oil treatment was approximately fivefold greater than that of the unsupplemented diet (Kelly *et al*. 1998a).

Bell and Kennelly (2002) reported that feeding lactating Holstein cows high-fat diets (details of the diets were not given because of patent confidentiality) increased *cis*-9, *trans*-11 CLA to 5.63% compared with 0.49% in the control, 0.56% in the low-fat diet and 3.7% in another high-fat diet. Although the milk fat yield was lower in the high-fat diet, the CLA yield (g/day) also increased to 45.8 compared with 5.4, 5.4 and 28.5, respectively. The high-fat diet also resulted in a significant increase in the *trans*-11$C_{18:1}$ content in the milk fat (Bell & Kennelly 2002; Table 2).

Changes in the roughage : concentrate ratio of the diet also influences the CLA content in milk. Kelly and Bauman (1996) observed that the CLA content in milk decreased to approximately 50% when its ratio was changed from 50:50 to 20:80. Bauman and Griinari (2001) showed that feeding a high concentrate/low roughage diet caused a shift in the biohydrogenation pathways with alterations in bacterial populations so that *trans*-10$C_{18:1}$ replaced *trans*-11$C_{18:1}$ as the predominant *trans*-$C_{18:1}$ in the milk fat. A putative pathway for

Table 1 Fatty acid composition of fresh grass, hay and soybean oil

Fatty acid	Fatty acid (%)		
	Fresh grass	Hay	Soybean oil
$C_{16:0}$	21.6	33.3	11.4
$C_{18:0}$	1.6	2.9	4.1
cis-9$C_{18:1}$	2.3	3.2	24.1
$C_{18:2}$ (linoleic acid)	15.5	17.8	50.9
$C_{18:3}$ (α-linolenic acid)	48.2	24.9	6.2
Others	10.9	18.0	3.3
$C_{18:2} + C_{18:3}$	63.7	42.7	57.1
Total fatty acid content (mg/g dry matter)	20.0	6.8	

Table 2 Fatty acid composition of milk fat from cows fed different diets (adapted from Bell and Kennelly (2002))†

Fatty acid	CTL	LF	HFA	HFB
$C_{18:0}$	6.25[a]	5.68	9.92[b]	8.75[c]
$C_{18:1}$*trans*-11	1.52[a]	1.65[a]	10.55[b]	14.77[c]
$C_{18:1}$*cis*-9	12.65[a]	13.16[a]	20.41[b]	18.25[c]
$C_{18:1}$ (n-12)	0.77[a]	1.05[a]	2.08[b]	1.64[c]
$C_{18:1}$ (n-7)	0.67[a]	0.69[a]	0.95[b]	0.91[b]
$C_{18:1}$ (n-6)	0.45[a]	0.52[a]	3.05[b]	2.30[c]
$C_{18:2}$	1.51[a]	1.62[a]	2.97[b]	2.82[b]
Cis-9, *trans*-11 CLA	0.49[a]	0.56[a]	3.70[b]	5.63[c]
Trans-10, *cis*-12 CLA	ND[a]	ND[a]	0.054[b]	0.054[b]
Trans/trans CLA	0.033[a]	0.046[a]	0.15[b]	0.17[b]
Total CLA yield (g/day)	5.1[a]	5.4[a]	28.5[b]	45.8[c]

†All values are presented as a percentage of fatty acid methyl esters. [abc]Within a row, values with different superscript letters are significantly different ($P < 0.05$). CLA, conjugated linoleic acid; CTL, control; LF, low-fat diet; HFA, high-fat diet A; HFB, high-fat diet B.

the formation of *trans*-10$C_{18:1}$ is shown in Fig. 2. The initial reaction involves isomerization of the *cis*-9 double bond to form an intermediate with *trans*-10, *cis*-12 conjugated double bonds. In the case of linoleic acid, this intermediate is *trans*-10, *cis*-12 CLA. Consequently, both the *trans*-10$C_{18:1}$ and *trans*-10, *cis*-12 CLA content in the milk increased with high concentrate/low roughage diets (Bauman & Griinari 2001). The biological effects of this CLA isomer on fat synthesis is described below.

Whitlock *et al*. (2002) found that extruded soybeans and fish oil fed alone or in combination also increased the total CLA levels (mg/g fat) to 11.8, 20.7 and 18.6, respectively, compared with the control (6.0). Additional factors determining the CLA level in the milk fat are the breed (Lawless *et al*. 1999), the age of the animal (Peterson *et al*. 2002) and the season (Stanton *et al*. 1997). The CLA level in milk fat changes throughout the year, being significantly higher in May, June and July (Lock & Garnsworthy 2003) or during pasture feeding (Jahreis *et al*. 1997; Precht & Molkentin 1999).

The synthetics produced from vegetable oils (safflower or sunflower oil) contain numerous CLA isomers. These CLA are protected by producing calcium salts (Giesy *et al*. 1999) or by the formaldehyde treatment of CLA encapsulated in a casein matrix (Gulati *et al*. 2000). Apparent transfer efficiencies for CLA isomers vary from 22 to 34% for abomasal infusions in cows (Chouinard *et al*. 1999), and from 36 to 41% in goats fed protected CLA (Gulati *et al*. 2000). However, the synthetic CLA infusion into the abomasum in dairy cows results in a dramatic reduction in milk fat yield. Baumgard *et al*. (2000) infused a relatively pure CLA isomer to the abomasum in dairy cows. After 4 days of infusion in dairy cows, *trans*-10, *cis*-12 CLA resulted in a more than 40% reduction in milk fat percentage and yield, whereas *cis*-9, *trans*-11 CLA and the control (safflower oil) had no effect (Fig. 3). Griinari *et al*. (1999) also observed a curvilinear relationship between the increase in the *trans*-10, *cis*-12 CLA dose and the reduction in milk fat yield (Fig. 4), and they suggested that this isomer has a potent inhibitory effect on milk fat synthesis.

The CLA content in milk fat may be influenced by the ratio of forage : concentrate with a constant supply of linoleic acid (Jiang *et al*. 1996). As previously mentioned, feeding a high concentrate/low roughage diet results in bacterial population shifts that alter the pattern of fermentation end products. The shift in rumen bacteria populations modifies the biohydrogenation pathway. As shown in Fig. 2, the initial reaction involves isomerization of the *cis*-9 double bond to form an intermediate with *trans*-10, *cis*-12-conjugated double bonds. The intermediate of linoleic acid is *trans*-10, *cis*-12 CLA (Griinari *et al*. 1999). Bauman and Griinari (2001) suggested that the rumen environment that leads to the formation of *trans*-10$C_{18:1}$ and *trans*-10, *cis*-12 CLA is likely to result in other unique biohydrogenation intermediates that inhibit milk fat synthesis, and they referred to it as the "biohydrogenation theory" of milk fat depression.

Thus, a protected CLA supplement appears to meet our expectations for increasing CLA in dairy products, so long as the economics of supplementation balances the added value of the products.

Physiological functions and metabolism of conjugated linoleic acid

Prior to 1987, scientific interest in CLA was confined largely to rumen microbiologists who studied the *cis*-9, *trans*-11 CLA isomer as an intermediate in the biohydrogenation of linoleic acid. This changed when Ha *et al*. (1987) reported that CLA produced by base-catalyzed isomerization of linoleic acid was an effective inhibitor of benzopyrene-initiated mouse epidermal neoplasia. Since then, CLA has gained considerable attention as a nutrient that exerts the following beneficial physiological effects in experimental animals and human beings: (i) inhibition of carcinogenesis; (ii) prevention of cholesterol-induced atherosclerosis (reduction of LDL concentration and the ratio of LDL : high-density lipoprotein (HDL)); (iii) reduction of bodyfat accumulation (reduced whole bodyfat); (iv) enhancement of the immune response; (v)

ability to enhance growth promotion (increased body protein); (vi) improvement of diabetes (the normalization of impaired glucose tolerance); and (vii) improvement of bone metabolism.

Effects of conjugated linoleic acid on carcinogenesis

Pariza *et al*. (1983) took extracts from grilled ground beef and found that they had "mutagen modulators" (before they isolated CLA) and acted as cancer inhibitors. Ip *et al*. (1996) reported that 0.05–0.50% dietary CLA reduced the mammary tumor yield when fed to rats treated with 7, 12-dimethylbenz anthracene, a known cancer-causing substance (Fig. 5). Furthermore, Bauman *et al*. (2000) produced CLA-enhanced butter by dietary manipulation of dairy cows, and compared it with two chemically synthesized CLA sources in a rat mammary cancer model (Ip *et al*. 1999). Animals fed diets with CLA, including CLA-enriched butter diets, had a lower tumor incidence and fewer mammary tumors than those fed control diets (Table 3). The *cis*-9, *trans*-11 CLA isomer represented 91% of the total CLA in the CLA-enriched butter, further demonstrating that this isomer is anticarcinogenic. These results are particularly exciting because they are the first to demonstrate that a natural anticarcinogen as a component of a natural food effectively reduces tumors in an animal cancer model. Thereafter, there have been an increasing number of reports on CLA in milk. CLA seems to inhibit carcinogenesis by mechanisms directly affecting the stages of carcinogenesis known as initiation (Ha *et al*. 1987; Liew *et al*. 1995), tumor promotion (Ip *et al*. 1994, 1996), progression and metastasis (Cesano *et al*. 1998). CLA may also indirectly affect the onset of cancer by reducing excessive bodyfat accumulation, which indirectly influences the risk of cancer (Pariza 1999). CLA may reverse the progression of cancer by reducing cachexia, which is associated with advanced cancer. Cachexia is mediated by cytokines, especially tumor necrosis factor-α (Hotamisligil & Spiegelman 1994).

It is well known that when provided in the diet or as supplemental oil, CLA isomers accumulate in the tissues of animals and humans (Belury 1995). In addition, isomers of CLA are readily metabolized to conjugated arachidonic acid via multiple metabolic pathways *in vivo* (Fig. 6). Elongated and desaturated metabolites of CLA have been identified in many

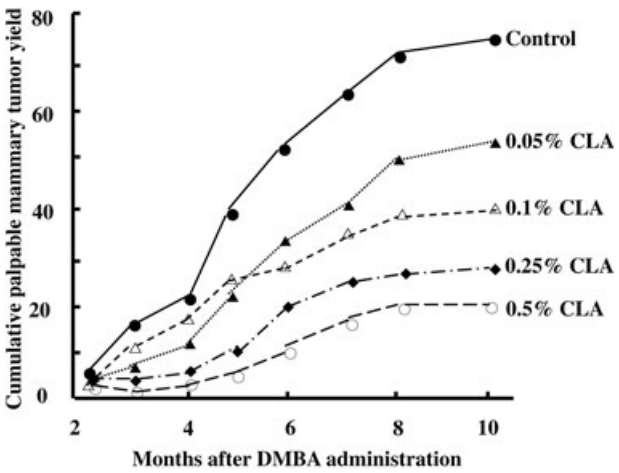

Fig. 5 Cumulative appearance of palpable mammary tumors as a function of time after the administration of dimethylbenz-α anthracene (DMBA) in rats fed different levels of conjugated linoleic acid (CLA). After each CLA diet was fed to 50 rats for 2 weeks, DMBA was given orally (adapted from Ip *et al.* (1996)).

Table 3 Incidence of mammary tumors in rats fed diets containing different concentrations and sources of conjugated linoleic acid (CLA) and treated with a chemical carcinogen (adapted from Ip *et al*. (1999))†

Treatment group	Dietary CLA (g/100 g)	Tumor incidence	Tumor numbers
Control	0.1	28/30 (93%)	92
CLA-enriched butter	0.8	15/30 (53%)	43
CLA supplement 1	0.8	16/30 (53%)	46
CLA supplement 2	0.8	17/30 (57%)	48

†Rats were fed treatment diets for 4 weeks and then given a single dose of a carcinogen (methyl-nitrosourea). After carcinogen administration, rats were fed a diet containing maize oil without CLA and were killed 24 weeks later. Supplement 1, *cis*-9, *trans*-11 CLA; supplement 2, mixture of CLA isomers (8–10, 9–11, 10–12, 11–13).

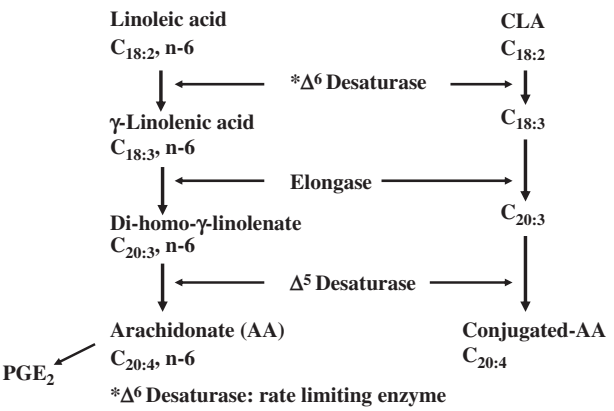

Fig. 6 Metabolism of linoleic acid and conjugated linoleic acid (CLA).

tissues in animals (Banni *et al*. 2001; Belury 2002). The discovery that CLA can be elongated and desaturated as linoleic acid to conjugated $C_{18:3}$, $C_{20:3}$ and $C_{20:4}$ indicates a new possibility that functions of CLA might be related to its metabolism, and possible competition with the other PUFA families, and in particular with *n*-6, for the formation of arachidonic acid (Banni *et al*. 1999). Isomers and metabolites of CLA are readily incorporated into phospholipid and neutral lipid fractions of numerous tissues like most other dietary PUFA (Ip *et al*. 1991, 1996; Belury & Kempa-Steczko 1997). However, a recent study on rats fed a diet containing CLA-rich butter and linoleic acid showed that the accumulation of CLA and linoleic acid into rat liver is not similar (Banni *et al*. 2001). In fact, CLA preferentially accumulated in neutral lipid (~79%) with less incorporation into phosphatidylcholine (~10%), the major phospholipid of hepatocytes. In contrast, linoleate accumulated preferentially in phosphatidylcholine (~50%), with less in neutral lipids (~17%). Furthermore, it was reported that conjugated $C_{18:3}$ and $C_{20:3}$ were incorporated similarly to CLA and very differently from their non-conjugated parent compounds $C_{18:3}$ (*n*-6) and $C_{20:3}$ (*n*-6). In contrast, conjugated $C_{20:4}$ was mainly incorporated into phospholipid (Banni *et al*. 2001). The preferential incorporation of conjugated $C_{18:3}$ and $C_{20:3}$ into the neutral lipid fraction allow these metabolites to be stored in the adipose tissue. This may provide an advantage in the competition with arachidonic acid because its precursors $C_{18:3}$ (*n*-6) and $C_{20:3}$ (*n*-6) are mostly incorporated into phospholipid. Interestingly, in mammary and adipose tissues, it seems that the decrease of 20 carbon atom PUFA, that is, $C_{20:4}$ and $C_{20:3}$ substrates of cyclooxygenase (COX-2) and lipoxygenase pathways, are replaced by the CLA isomers conjugated $C_{18:3}$ and $C_{20:3}$ (Banni *et al*. 1999), which have been demonstrated to inhibit both pathways of eicosanoid biosynthesis (Nugteren & Christ-Hazelhof 1987). It is likely that one mechanism by which CLA exerts many physiological functions is by modulating the accumulation of arachidonic acid in phospholipids, resulting in a reduced arachidonic acid pool and reduced production of downstream eicosanoid products (PGE_2) (Fig. 7). In fact, the role of CLA in the reduction of COX-2 activity has been demonstrated *in vivo* in the bone and macrophages. PGE_2 exhibits physiological functions as shown in Fig. 7. An excess of PGE_2 seems to promote a carcinogenic response, whereas a low level of PGE_2 appears to modulate immune functions and inhibit carcinogenic activity. Furthermore, an elongation product of *cis*-9, *trans*-12 CLA, conjugated eicosadienoic acid, reduces the proliferation of three cancer lines *in vitro* (Palombo *et al*. 2002).

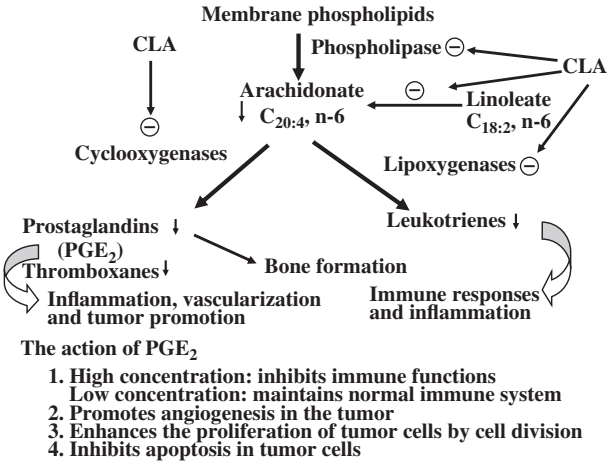

Fig. 7 Effect of conjugated linoleic acid (CLA) on downstream eicosanoid (PGE_2) production and the action of PGE_2.

As a counterbalancing event in promotion, apoptosis offers protection against carcinogenesis via the programmed death of cancer cells. Dietary CLA induces apoptosis in numerous tissues (Ip *et al*. 2000; Tsuboyama-Kasaoka *et al*. 2000) and in cultured mammary epithelial cells (Ip *et al*. 1999). In these studies, CLA induction of apoptosis was associated with a reduction of bcl-2, a signaling protein known to suppress apoptosis. These data suggest that CLA may inhibit promotion by inducing signaling events leading to enhanced apoptosis.

Conjugated linoleic acid induces markers of differentiation in a non-cancer model, adipose tissue (Houseknecht *et al*. 1998; Satory & Smith 1998). Therefore, it is possible that CLA inhibits carcinogenesis via the induction of differentiation. In fact, the finding that CLA fed during the time of mammary tissue development and maturation has long-lasting protective effects against mammary carcinogenesis (Ip *et al*. 1994; Thompson *et al*. 1997), suggesting that the role of CLA in protecting against mammary carcinogenesis may be, in part, by modulating tissue differentiation.

Effects of conjugated linoleic acid on atherosclerosis

A high cholesterol level in the plasma has been ranked as one of the greatest risk factors in the development of chronic heart disease (Grundy 1986). CLA reduces LDL cholesterol in the plasma and inhibits the development of atherosclerosis in hamsters (Nicolosi

et al. 1997) and rabbits (Lee *et al*. 1994). Thus, the risk factors for atherosclerosis, including the LDL-cholesterol : HDL-cholesterol ratio and the total cholesterol : HDL-cholesterol ratio, are significantly reduced. It appears that the retardation of atherosclerosis by CLA is, at least in part, a result of changes in lipoprotein metabolism. Alteration of the lipoprotein profile in the plasma is also an important factor in the disease.

Atherosclerosis is a disease of the intima characterized by the accumulation of smooth muscle cells and lipid. There is evidence that CLA reduces atherosclerotic plaque formation in hamsters (Wilson *et al*. 2000). Kritchevsky *et al*. (2000) reported that rabbits fed CLA had significantly reduced atherosclerotic involvement in both the aortic arch and thoracic aorta (Fig. 8). The percentage of esterified cholesterol present in the aortic tissue can be used as a measure of the severity of atherosclerosis, and cholesterol ester was reduced in rabbits fed the CLA diet.

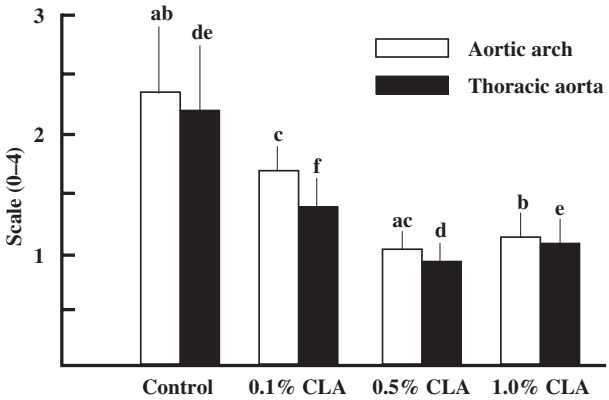

Fig. 8 Conjugated linoleic acid (CLA) and involution of atherosclerosis in rabbits (adapted from Kritchevsky *et al*. (2000)).

One of the possible mechanisms by which dietary CLA decreases serum cholesterol has been shown by Thomas *et al*. (2000). When hamsters were fed a semi-synthetic diet containing 1 g cholesterol/kg diet with or without supplementation with a 20 g linoleic acid and 20 g CLA/kg diet for 8 weeks, serum fasting total cholesterol and triglyceride were significantly lower in the linoleic acid-supplemented and CLA-supplemented groups than in control hamsters. The addition of dietary CLA resulted in a decrease in the activity of intestinal acyl : cholesterol acyltransferase (ACAT), whereas linoleic acid had no effect on this enzyme (Fig. 9). ACAT functions mainly to esterify cholesterol and store it as cholesterol ester; thus, it may be involved in the intestinal absorption of cholesterol. There is evidence that the majority of dietary cholesterol is esterified before it is assembled in chylomicrons and secreted into the lymphatic system (Wrenn *et al*. 1995). Dietary CLA may interfere with the absorption of cholesterol by inhibiting ACAT activity and thereby increasing the neutral sterol excretion in hamsters (Fig. 9). Therefore, the reduction in serum cholesterol by dietary CLA is likely to be mediated, in part, by its inhibitory effect on cholesterol absorption via the decrease in intestinal ACAT activity. It remains unclear whether dietary CLA reduces serum lipids in human participants.

Effects of conjugated linoleic acid on lipid metabolism

Conjugated linoleic acid has been shown to decrease bodyfat while not affecting total body mass. Pigs fed

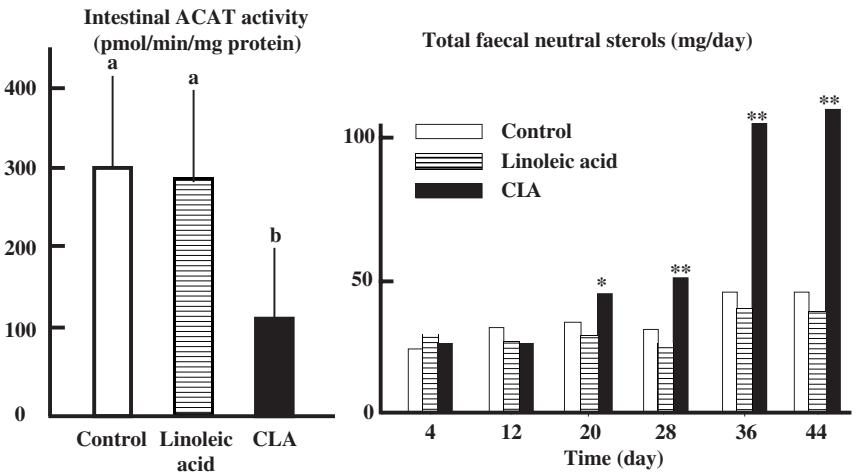

Fig. 9 Effects of conjugated linoleic acid (CLA) and linoleic acid on intestinal acyl coenzyme A : cholesterol acyltransferase (ACAT) activity and fecal output of total neutral sterols in hamsters. Hamsters were fed semi-synthetic diets containing 0.1% cholesterol with or without 2% LA and 2% CLA for 8 weeks (adapted from Thomas *et al*. (2000)). a,b, $P < 0.05$; *$P < 0.05$; **$P < 0.01$.

CLA develop less bodyfat and show improved feed conversion efficiency (Dugan *et al*. 1997). CLA reduces the carcass fat content in mice (Belury & Kempa-Steczko 1997; West *et al*. 1998) and reduces the backfat thickness in pigs (Cook *et al*. 1998). Dietary CLA supplements increase lean tissue deposition and decreases fat deposition in pigs (Ostrowska *et al*. 1999). The mechanism by which CLA leads to a decrease in fat deposition is shown in Fig. 10.

Conjugated linoleic acid seems to cause a loss of appetite in human subjects. The activity of carnitine palmitoyltransferase, the rate-limiting enzyme for fatty acid β-oxidation, also increases (Park *et al*. 1997). Furthermore, in a related *in vitro* experiment (Park *et al*. 1997), it was observed that adding CLA to the media of 3T3-L1 adipocytes (at levels found in the blood of rats fed 0.5% CLA) significantly reduces the activity of lipoprotein lipase (LPL), an enzyme involved in the uptake of fatty acids into adipose tissues from the blood, and reduces intracellular concentrations of triglyceride and glycerol. CLA treatment also enhances the release of fatty acids from these cells into the media. The latter observation was interpreted as indicating that CLA may enhance lipolysis. Therefore, the decrease in bodyfat associated with CLA may be partially a result of reduced fat deposition and increased lipolysis in adipocytes, possibly coupled with increased fatty acid oxidation in both skeletal muscle cells (the principal site of fatty acid oxidation) and adipocytes (the principal site of fat storage). Pariza *et al*. (2001) reported a model for the effects of CLA on adipocytes as shown in Fig. 11.

Effects of CLA on lipid metabolism may be mediated by effects of *trans*-10, *cis*-12 CLA. Park *et al*. (1999) reported that changes in the body composition (reduced bodyfat content and enhanced body protein) were observed in mice fed a diet with *trans*-10, *cis*-12 CLA. *Trans*-10, *cis*-12 CLA resulted in a decrease in LPL activity, and a decrease in levels of intracellular triglyceride and glycerol in 3T3-L1 adipocytes. However, *cis*-9, *trans*-11 CLA did not cause these effects.

The data shown in Table 4 indicate that *cis*-9, *trans*-11 CLA is active in enhancing bodyweight gain and also appears to enhance feed efficiency in weanling mice. However, *cis*-9, *trans*-11 CLA has no effect on bodyfat levels. In contrast, *trans*-10, *cis*-12 CLA reduces bodyfat levels relative to the control but do not enhance either body growth or feed efficiency. It is considered that *trans*-10, *cis*-12 CLA reduces the uptake of lipids by adipocytes, and that *cis*-9, *trans*-11 CLA is active in inhibiting carcinogenesis. Effects of *trans*-10, *cis*-12 CLA appear to be related to the observation that abomasal infusion of *trans*-10, *cis*-12 CLA results in an immediate decrease in milk fat synthesis, and a role for *trans*-10, *cis*-12 CLA as a specific inhibitor of milk fat synthesis has been proposed (Griinari *et al*. 1998). Dairy breed variations in milk fat CLA content may be related to differences in Δ^9-desaturase activity (Beaulieu & Palmquist 1995; DePeters *et al*. 1995).

The effects of CLA isomers on metabolites are different. Sebedio *et al*. (2001) demonstrated that *cis*-9, *trans*-11 CLA is converted into $C_{18:3}$ and $C_{20:3}$ conjugated fatty acids as shown in Fig. 6. *Trans*-10, *cis*-12 CLA is converted mainly into delta 6, 10 and 12, whereas only smaller quantities of the conjugated $C_{20:3}$ delta 8, 12 and 14 have been detected. Only a small quantity of conjugated $C_{20:4}$, formed from *trans*-10, *cis*-12 CLA, has been found in phospholipid. Furthermore, *trans*-10, *cis*-12 CLA can be converted into a conjugated

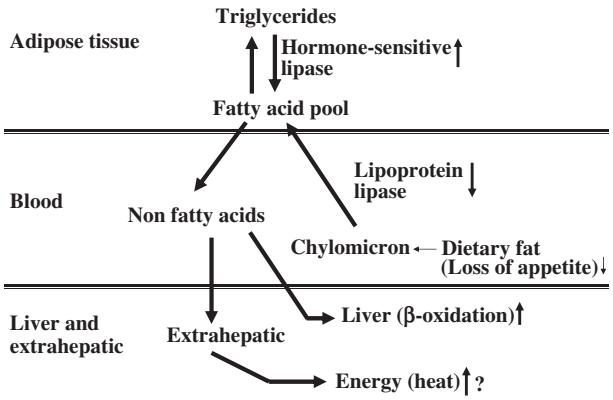

Fig. 10 Conjugated linoleic acid and lipid metabolism.

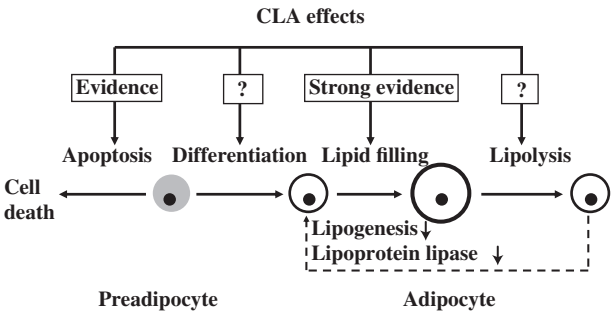

Fig. 11 A model for the effects of trans-10, cis-12 conjugated linoleic acid (CLA) on adipocytes and preadipocytes (adapted from Pariza *et al*. (2001)).

Table 4 Independent effects of dietary *cis*-9, *trans*-11 conjugated linoleic acid (CLA) or *trans*-10, *cis*-12 CLA on growth, feed conversion and bodyfat levels in mice (adapted from Cook *et al*. (2000))[†]

cis-9, *trans*-11 (g/100 g diet)	*trans*-10, *cis*-12 (g/100 g diet)	0–3 week bodyweight gain	0–3 week feed conversion (gain/feed intake)	Bodyfat (%)
0.0 (Control)	0.0	9.9	0.125	14.0
0.1	0.0	9.8[‡]	0.125	9.9
0.2	0.0	13.2[‡]	0.156	13.2
0.4	0.0	11.6[‡]	0.133	13.4
0.0	0.1	9.4	0.118	8.8[§]
0.0	0.2	8.9	0.114	6.4[§]
0.0	0.4	9.4	0.112	3.0[§]

[†]Weaning mice (6/group) were fed the control diet, a diet supplemented with *cis*-9, *trans*-11 CLA, or a diet supplemented with *trans*-10, *cis*-12 CLA. [‡]The trend in bodyweight gain for the groups fed *cis*-9, *trans*-11 was significantly different from the control ($P < 0.03$). [§]Percentage bodyfat was significantly reduced relative to controls in the groups fed *trans*-10, *cis*-12 CLA ($P < 0.0001$).

$C_{16:2}$ that is found only in liver lipid from animals fed *trans*-10, *cis*-12 CLA. This may suggest that *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA are metabolized differently through the peroxisomal β-oxidation pathway.

In addition to the beneficial health effects already described, there is evidence showing that CLA may exhibit important physiological functions, such as antidiabetic properties, immune modulation and improved bone metabolism, although the detailed mechanisms are not clear. The main dietary source of CLA is ruminant products, and in order for humans to obtain a significant intake of CLA in natural foods, ruminant products are the best ones available.

SUMMARY

The presence of CLA in the milk and adipose tissue (bodyfat) from ruminants relates to the biohydrogenation of PUFA by rumen bacteria. Ruminant fat contains numerous isomers of CLA. *Cis*-9, *trans*-11 CLA comprises 75–90% of total CLA, and although it is an intermediate in ruminal biohydrogenation of linoleic acid, its major source is endogenous synthesis involving Δ^9-desaturase with *trans*-11$C_{18:1}$ produced in the rumen as the substrate. Thus, the keys to increase the CLA content of ruminant fat are to increase rumen output of *trans*-11$C_{18:1}$ and tissue activity of Δ^9-desaturase. The *trans*-10, *cis*-12 CLA isomer exerts specific effects on adipocytes. A model is presented in which this isomer acts primarily by reducing the uptake of lipid into adipocytes by inhibiting the activities of adipocyte LPL and Δ^9-desaturase. The *trans*-10, *cis*-12 CLA isomer also affects lipid metabolism in ruminants. Specifically, this isomer is a very potent inhibitor of milk fat synthesis in dairy cows, whereas the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers appear to actively inhibit carcinogenesis in animals. It is probable that some of the effects of CLA are a result of CLA-induced changes in linoleic acid metabolism. It is likely, but not yet proved, that metabolites (e.g. conjugated $C_{20:3}$ and $C_{20:4}$) of CLA isomers are involved in some aspects of the biochemical mechanisms whereby CLA induces its physiological effects. At least in experimental animals, CLA has been clearly shown to be effective as a preventive agent against different pathologies.

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