# Potential Health Benefits of Conjugated Linoleic Acid (CLA): A Review

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**ABSTRACT :** Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of octadecadienoic acid with two conjugated double bonds. Of more than a dozen isomers of CLA found naturally in dairy and meat products from ruminants, *c*-9, *t*-11 and *t*-10, *c*-12 are the two isomers with known physiological importance, including anticarcinogenic, antidiabetic, antilipogenic, and antiatherosclerotic effects. Positive effects of CLA on immune function and bone modeling have also been reported. In spite of the compelling findings in tissue cultures and experimental animal models, its effect, dose, and mechanism of action vis-à-vis specific isomers remains speculative. Results obtained from animal models are inconclusive and conflicting at times in humans, where the research data is limited. It appears that there is a long way to go before CLA could be accepted unequivocally as having definite effects in any or all of these physiological states and how such effects actually occur in humans. The objective of this review is to critically examine the available literature on potential health benefits of CLA observed in cell culture, animal models, and human subjects, wherever possible and to a certain extent the mechanism of action associated with these biological activities. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1315-1328)***

**Key Words** : Conjugated Linoleic Acid, Potential Health Benefits, Animals, Humans

## INTRODUCTION

Conjugated linoleic acid (CLA), found naturally in food products from ruminants, refers to a mixture of positional and geometric isomers of linoleic acid (*c*-9, *c*-12 $C_{18:2}$) with two conjugated double bonds at various carbon positions in the fatty acid (FA) chain. Each double bond can be *cis* or *trans*, but those with one *trans* double bond are bioactive (Jensen, 2002). It is formed as an intermediate during the biohydrogenation of linoleic acid to stearic acid in the rumen by *Butyrivibrio fibrisolvens* (Kepler et al., 1966) and other rumen bacteria (Kritchevsky, 2000) or from the endogenous conversion of *t*-11 $C_{18:1}$ (transvaccenic acid, TVA), another intermediate of linoleic or linolenic acid biohydrogenation, by $\Delta^9$-desaturase in the mammary gland (Griinari and Bauman, 1999; Corl et al., 2001) and possibly in adipose tissues (Gillis et al., 2003). Of the two physiologically important isomers, *c*-9, *t*-11 is the most prevalent one comprising 80 to 90% of total CLA in foods from ruminants (Table 1), whereas *t*-10, *c*-12 isomer is present in small amounts at 3 to 5% of the total. Another isomer *c*-9, *c*-11 has been found even more potent that *c*-9, *t*-11 or *t*-10 *c*-12 isomers against human breast cancer cells recently (Tanmahasamut et al., 2004). However, its presence in lipids from ruminants is rarely reported. Although it is relatively easy to raise the concentration of *c*-9, *t*-11 CLA in ruminant lipids primarily through manipulation of animal diet, increase in *t*-10, *c*-12 isomer content through manipulation of animal diet is nominal (Khanal and Olson, 2004). Based on the dose required in animal models (Ip et al., 1999), such an approach would help meet the dose shown to be effective to derive the potential health benefits associated with *c*-9, *t*-11, but not *t*-10, *c*-12 CLA.

Ever since Ha et al. (1987) demonstrated that CLA obtained from fried ground beef inhibited carcinogenesis, a whole new era of research dedicated to CLA has opened. Initially the studies were related more to the anticarcinogenic properties of CLA. Later its positive effects on diabetes (Belury and Vanden Huevel, 1999), atherosclerosis (Nicolisi et al., 1997), lipid metabolism (Terpstra et al., 2002), bone modeling (Watkins and Seifert, 2000), immune response (Cook et al., 1999), and vitamin A metabolism (Carta et el., 2002) have been demonstrated in various experimental animal models. Although there have been a few attempts at verifying the positive effects of CLA in human health through case control studies, it is not yet possible to clearly state that CLA supports all those benefits in humans as well. Moreover, the mechanisms of action through which CLA exerts its effects have been speculative at best (Belury, 2002b; Pariza et al., 2003). Another important question that has been raised but not answered is the dose needed to bring about the desired effects vis-à-vis the specific CLA isomers. Some of the reviews on the subject have dealt the beneficial effects of CLA on these physiological cases, e.g. cancer (Banni et al., 2003), diabetes (Belury, 2003), lipid metabolism (Keim, 2003), bone modeling (Watkins et al., 2003), immune response (Cook et al., 2003) etc., separately in detail, though sometime with a thinking that CLA could be beneficial in everything related to obesity. The objective of this paper is to provide a critical and comprehensive review on various aspects of CLA's potential health benefits and the

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**Table 1.** The mean positional and geometric isomer composition (% of total isomers) and the CLA content of samples of milk, butter, cheese, and beef fat[1]

| CLA isomer | Milk[2] | Butter | Cheese | Beef |
|---|---|---|---|---|
| *cis, trans*-isomers | | | | |
| 7, 9 | 5.5 | 6.7 | 3.6 | 7.0 |
| 8, 10 | 1.5 | 0.3 | 1.0 | 2.6 |
| 9, 11 | 72.6 | 76.5 | 83.5 | 72.0 |
| 10, 12 | 0.4 | 1.1 | - | 2.6 |
| 11, 13 | 7.0 | 0.4 | 4.7 | 1.1 |
| 11, 13 | - | - | - | 2.2 |
| 12, 14 | 0.7 | 0.8 | 0.4 | 0.7 |
| Total *cis, trans* (*trans, cis*) | 87.7 | 85.8 | 93.2 | 88.2 |
| *Trans, trans* isomers | | | | |
| 6, 8 | | - | 0.1 | 0.7 |
| 7, 9 | 2.4 | - | 0.6 | 1.5 |
| 8, 10 | 0.4 | - | 0.3 | 0.7 |
| 9, 11 | 2.0 | - | 1.5 | 3.7 |
| 10, 12 | 0.6 | - | 0.5 | 1.9 |
| 11, 13 | 4.2 | - | 2.3 | 1.9 |
| 12, 14 | 2.8 | - | 0.9 | 1.9 |
| 13, 15 | - | - | 0.1 | - |
| Total *trans, trans* | 12.3 | 9.4 | 6.3 | 12.3 |
| *Cis, cis* isomers | | | | |
| 8, 10 | - | - | <0.1 | - |
| 9, 11 | - | - | 0.3 | - |
| 10, 12 | - | - | <0.3 | - |
| 11, 13 | - | - | 0.3 | - |
| Total *cis, cis* | - | 4.8 | 0.7 | - |
| Total CLA (% of fat) | | 0.5 | 0.93 | 0.27 |

[1] Adapted from Parodi (2003). [2] Shingfield et al. (2003).

mechanisms of action speculated thus far in a rather brief form without having to make a case for CLA.

**CLA and cancer**

Fats in general have been implicated in many forms of cancer, yet evidence is accumulating that certain types of FA have anticancer properties, of which CLA is the major one. Inhibitory effects of CLA against carcinogenesis have been demonstrated in a variety of cell type, sites, and animal models including mammary gland, skin, colon, prostate, and forestomach of rats, humans, mice, and hamsters. In contrast to the hundreds of phytochemicals possessing varying degrees of anticancer properties (Scimeca, 1999), CLA is unique in that it is a FA, is found in highest amounts in food products derived from ruminants, and is safe at dietary levels. It is believed that CLA is involved in a variety of biological events in all three stages of carcinogenesis viz initiation, promotion, and progression. It is also believed that the effects vary with the specific isomers of CLA and the type and site of the cell/organ as well as the stage of tumerogenesis. Overall, the effects of CLA are related to inhibition of growth and proliferation, induction of apoptosis, and diminishing branching and reducing the density of ductal system of the cancerous cells (reviews by Kritchevsky, 2000; Parodi, 2001; Belury, 2002a). Some important and recent studies related to CLA and cancer has been presented in Table 2. For further reviews on the subject one is referred to Banni et al. (2003), Pariza et al. (2003), Scimeca (1999), and various references cited in Table 2. It should, however, be mentioned that none of these effects have been verified by clinical studies in humans.

It was found that mammary tumor mass (Lavillonniere et al., 2003) and incidence and weight (Ip et al., 1991) was reduced in rats fed CLA. Exposure to 1% CLA during the early preweaning and pubertal period only was sufficient to reduce the subsequent methylnitrosurea induced tumerogenesis in rats (Ip et al., 1995). It may have further implications in cancer prevention in humans once it is proven with clinical case-control studies, because preweaning and pubertal period are two different stages in mammary development. Ip et al. (1999) also found that CLA decreased mammary tumor incidence by 50% and tumor number by 45% in rats fed CLA at 0.8% of the diet. By way of comparison, the efficacy of fish oil, which is also an anticancer agent and which is not plant derived, is 100 times lower than that of CLA (Scimeca, 1999). Hubbard et al. (2000) showed that CLA was not only involved in altering mammary tumor incidence, but also effect later stages, especially metastasis as effectively as indomethacin, a known suppressor of tumor growth and metastasis in murine mammary tumors. It supports the notion that CLA may be efficacious in preventing the development and recurrence of some cancers as well as suppressing the growth of residual disease. Moreover, effect of CLA as an anticancer agent is same whether it is provided through high CLA foods produced naturally or when it is supplied as pure synthetic CLA isomers (Ip et al., 1999). For instance, O'Shea et al. (2000) found that cell number was decreased up to 90% and lipid peroxidation increased by 15 fold following incubation of breast cancer cells for 8 days with increasing levels of milk yielding CLA concentrations between 16.9 and 22.6 ppm. Similarly, when human breast cancer cells were cultured with CLA enriched milk, cell number decreased to 61% of the original level (Miller et al., 2003b). An important observation was that rats fed CLA enriched butterfat accumulated more CLA in the mammary gland and other tissues than did rats fed synthetic CLA isomers (Ip et al., 1999). This led to a new hypothesis (discussed later) that TVA in butter fat may serve as the precursor for the endogenous synthesis of CLA by $\Delta^{-9}$ desaturase. These observations are encouraging and provide a venue for planning intervention strategies through diet. It should be noted that synthetic CLA contains about a dozen of isomers, *c*-9, *t*-11 and *t*-10, *c*-12 being the most abundant ones at approximately 1:1 ratio, while that present in milk, meat, or butter fat is primarily *c*-9, *t*-11 at 80 to 90% or

**Table 2**. Biological effects of CLA on carcinogenesis and diabetes

| Study/model/cell line | Effects | Isomer* |
|---|---|---|
| A. Carcinogenesis | | |
| Human breast cancer cells[1] | Damage to carcinogenic DNA | a |
| Caco-2 cells[2] | Reduced cell number and gene expression | b |
| Endotoxin-activated macrophage[3] | Suppression of COX-2 and NOS | d |
| Rat mammary cancer cells[4] | 33-36% reduction in number | a/b |
| DMBA and DMH treated rats[5] | Reduction in cell number and proliferation | d |
| Human SGC-7901 cell line[6] | Reduced invasion of SGC-7901 cells | a |
| Patients with localized breast cancer[7] | No effect | a |
| Human colon HT-29 cell line[8] | Reduced DNA and increased apoptosis | d |
| Epidemiological (breast cancer)[9] | Weak, positive relation | d |
| SGC-7901 cells *in vitro*[10] | Blocked cell cycle, increased apoptosis | a |
| Mouse forestomach neoplasia[11] | Increased apoptotic cells, reduced tumor size | a/b |
| Several[12] | Reduced carcinogen-DNA adduct formation | d |
| Rat mammary tumor[13] | Reduced size and number | a |
| Human case-control (breast cancer)[14] | -ve correlation with CLA and TVA | d |
| Finnish breast cancer study[15] | Inverse relation with the intake of milk and dairy products | - |
| Women with breast cancer[16] | Reduced tumors and tumor mass | d |
| Mice[17] | Decreased tumor yields | c |
| B. Diabete | | |
| Human diabetics[18] | Decreased blood glucose, plasma leptin, body weight and body mass index | b |
| Obese Zucker (fa/fa) rats[19] | Improved glucose tolerance and glucose transport | b |
| C57Bl/6J mice[20] | Induced hyperinsulinemia and fatty liver | b |
| Obese Zucker rat[21] | Improved glucose transport | a/b |
| Obese men[22] | Increased insulin resistance and glycemia | b |
| Zucker diabetic rats[23] | Increased glucose transport, glycogen synthase, and glucose tolerance and upregulated UCP2 | b |
| Type 2 diabetic subjects[24] | Inverse relation with body weight and serum leptin | b |

* a=*c*-9, *t*-11, b=*t*-10, *c*-12, a/b=*c*-9, *t*-11 and *t*-10, *c*-12, c=mixture, d=not known.

[1] Majumdar et al. (2002), [2] Kim et al. (2002a), [3] Iwakiri et al. (2002), [4] Ip et al. (2002), [5] Cheng et al. (2003), [6] Yang et al. (2003b), [7] Chajes et al. (2003), [8] Cho et al. (2003a), [9] Voorips et al. (2002), [10] Liu et al. (2002), [11] Chen et al. (2003), [12] Scimeca (1999), [13] Ip et al. (1999), [14] Aro et al. (2000), [15] Knekt and Järvinen (1999), [16] Lavillonniere and Bougnoux (1999), [17] Belury et al. (1996), [18] Belury et al. (2002), [19] Henriksen et al. (2003), [20] Clément et al. (2002), [21] Teachey et al. (2003), [22] Risérus et al. (2002), [23] Ryder et al. (2001), [24] Belury et al. (2003).

even more of the total CLA isomers.

Using the apoptotic marker YO-PRO(R)-1, Drouin et al. (2004) showed that *c*-9, *t*-11 CLA was the best apoptotic inducer compared with *t*-10, *c*-12 or a mixture of *c*-9, *t*-11 CLA and *t*-10, *c*-12 isomers both in MCF-7 and MDA-MB-231 cells. This is in contrast to previous findings on colorectal and prostate cancer cells where *t*-10, *c*-12 CLA led to an apoptotic response (Miller et al., 2002; Palombo et al., 2002). Moreover, *c*-9, *t*-11 CLA also turned out to be the best radiosensitizer compared with *t*-10, *c*-12 or a mixture of *c*-9, *t*-11 CLA and *t*-10, *c*-12 isomers (Drouin et al., 2004). The radiosensitizing property of *c*-9, *t*-11 CLA supports its potential as an agent to improve radiotherapy against breast carcinoma and needs further research for its use in humans.

At cytosolic concentrations, CLA elicited cell cycle arrest in G1 and induced the accumulation of the tumor suppressors p53, p27, and p21 protein, with *t*-10, *c*-12 isomer being more effective than *c*-9, *t*-11 isomer in inhibiting cell proliferation of MCF-7 breast cancer cells and enhancing the accumulation of p53 (Kemp et al., 2003). It reduced serum and mammary gland vascular endothelial growth factor (VEGF) and its receptor Flk-1 concentrations (Masso-Welch, 2002; Ip et al., 2003). Both *c*-9, *t*-11 and *t*-10, *c*-12 isomers inhibited functional vascularization of a matrigel pellet in vivo and decreased serum VEGF concentrations; the *t*-10, *c*-12 isomer also decreased the proangiogenic hormone leptin (Masso-Welch et al., 2004). Additionally, the *t*-10, *c*-12 isomer, but not *c*-9, *t*-11, rapidly induced apoptosis of the white and brown adipocytes as well as the preexisting supporting vasculature of the mammary fat pad, with both isomers inducing a rapid and reversible decrease in the diameter of the unilocular adipocytes (Masso-Welch et al., 2004).

In rodents, CLA-induced reduction in colon cancer incidents (Kim et al., 2002a) is probably through mechanisms involving apoptosis (Cho et al., 2003a). After 4 days of incubation with pure *c*-9, *t*-11 CLA, *t*-10, *c*-12 CLA and their mixture, CLA-treated colon SW 480 tumor cells displayed an increase in the activity of caspase-3 (27-34%) and caspase-9 (37-47%) (Miller et al., 2002), both of which are associated with apoptosis. Similar results of caspase-dependent apoptosis were observed in both colo-rectal and prostate cancer cells Palombo et al. (2002), which were also

time- and dose-dependent in Jurkat T cells (Bergamo et al., 2004). In human colo-rectal cells, both *c*-9, *t*-11 and *t*-10, *c*-12 CLA isomers induced dose-dependent inhibitory effects on cancer proliferation in vitro (Palombo et al., 2002). In human HT-29 colon cancer cell line, CLA inhibited cell proliferation and stimulated apoptosis in a dose-dependent manner by downregulating mRNA and protein levels of ErbB2 and ErbB3, both of which are implicated in the development of colon cancer (Cho et al., 2003b). In another study, *c*-9, *t*-11 CLA fed rats inhibited the development of aberrant crypts, also called preneoplastic lesions in colon cancer (Nichenametla et al., 2004), while *t*-10, *c*-12 CLA inhibited HT-29 cell growth through reduced IGF-II secretion (Cho et al., 2003b). In Caco-2 cell line, inhibition of growth by CLA has been proposed to be due in part to increased oleamide production (Kim et al., 2002a).

In human gastric carcinoma cell line, CLA inhibited the cell proliferation, cellular mitosis, cell clone formation and DNA synthesis, and induced cellular differentiation of SGC-7901 cells (Liu et al., 1999). In another study, *c*-9, *t*-11 CLA suppressed reconstituted basement membrane invasion of gastric carcinoma cell line SGC-7901 (Yang et al., 2003b). The growth and proliferation of SGC-7901 cells were inhibited by *c*-9, *t*-11 CLA via blocking the cell cycle and expression of bcl-2 pathways associated with mitochondria (Liu et al., 2002). In mouse forestomach neoplasia, both *c*-9, *t*-11 and *t*-10, *c*-12 isomers showed inhibited carcinogenesis in terms of the incidence and size of the tumor, mechanism for which was suggested to be complicated (Chen et al., 2003). With human bladder cancer cells, a decreased DNA synthesis and induced apoptosis was observed in a dose-dependent manner (Oh et al., 2003). In skin cancer in mice, CLA at 1.5% (wt/wt) reduced papilloma incidence from wk 8 to 24, whereas after 24 wk of tumor promotion tumor yield was inhibited at 1.0 or 1.5% (Belury et al., 1996), suggesting that inhibition of tumor promotion by CLA is independent of its antiinitiator activity.

In a few studies related to human subjects, Aro et al. (2000) observed a negative correlation of serum CLA and TVA with breast cancer risk in postmenopausal women. They found that the relative risk of breast cancer in these women with higher CLA intake was 0.3 relative to lower CLA intake, and when both CLA and TVA were combined, relative risk was reduced to 0.2. The authors hypothesized that a diet composed of CLA-rich dairy products may protect against breast cancer in postmenopausal women, even though it was not possible to assess the independent effects of CLA in the study. In another epidemiological study involving >25 years of follow-up in the Finnish breast cancer research, Knekt and Järvinen (1999) found an inverse relation between intakes of milk and dairy products with incidence of breast cancer and suggested that CLA could be the factor involved. In French women (N=360), number of tumors and incidence of tumor was less for CLA enriched diets than for control diets (Lavillonniere and Bougnoux, 1999). A proposition has been made that CLA specifically up-regulates cell signal systems at the level of gene expression (mRNA and protein) that are responsible for the induction of apoptosis in human breast and prostate cancer cells (Wahle and Heys, 2002). Although such findings are quite compelling, they cannot be taken as definitive. Further epidemiological, clinical, and intervention studies are needed to come to a definite conclusion before CLA can be used as an anticancer drug. It is more imperative because one Netherlands cohort study on diet and cancer in relation to postmenopausal breast cancer could not confirm the relationship of CLA with inhibition of cancer as has been observed in animal and tissue culture models (Voorips et al., 2002). Similarly no association between the level of CLA in the breast adipose tissue at the time of breast cancer diagnosis and the subsequent development of metastasis was observed in a cohort of 209 patients with an initially localized breast cancer (Chajes et al., 2003).

The effects of specific isomer(s) of CLA responsible for anticancer properties are not conclusive. Initially it was thought that *c*-9, *t*-11 CLA was responsible for the anticancer properties. However, *t*-10, *c*-12 has been found to be equally or even more potent than its *c*-9, *t*-11 counterpart (Ochoa et al., 2002; Ip et al., 2003), probably due to its antiadipogenic effects (Pariza et al., 2001; Terpstra et al., 2002) and cancer being related to obesity. Another study, however, showed that a mixture of *c*-9, *t*-11 and *t*-10, *c*-12 isomers was more potent than either of the two individual isomers (Majumdar et al., 2002). In yet another study, growth factor-induced proliferation of breast cancer cells were inhibited by both *c*-9, *t*-11 and *t*-10, *c*-12 isomers, with *c*-9, *t*-11 isomer exhibiting the strongest effect (Chujo et al., 2003). Recently, however, Tanmahasamut et al. (2004) showed *c*-9, *c*-11 to be more potent than either of the *c*-9, *t*-11 or *t*-10, *c*-12 isomers against human breast cancer cells. In human prostate cancer cell line, the antiproliferative effects of *c*-9, *t*-11 and *t*-10, *c*-12 CLA as well as their mixture were not equivalent and different pathways were involved for individual isomers (Ochoa et al., 2004). While *t*-10, *c*-12 isomer appeared to work preferentially through modulation of apoptosis and cell cycle control, *c*-9, *t*-11 worked through arachidonic acid metabolism (Ochoa et al., 2004). It is possible that the biopotency of individual isomers or their mixture depends on the cell type, organ, stage of tumerogenesis, and the species of animal used in the study.

It has been mentioned above that the mechanisms of action of CLA are not very clear, which may differ between the two major isomers concerned. It is also equally

reasonable to find different mechanisms in different cell types, stage of tumerogenesis, and species of animals. Potential mechanisms of action of CLA have been described in detail in recent reviews (Banni et al., 1999; Kritchevsky, 2000; Pariza et al., 2000; Banni et al., 2003; Pariza et al., 2003). Mechanism of action of CLA proposed thus far has been summarized below, but how the link between many of these potential mechanisms could be established to clearly define its role in various forms and stages of cancer remains to be further researched.

Triggers apoptosis through up-regulation of cell signal systems at the level of gene expression, both mRNA and protein (Wahle and Heys, 2002) or through oxidative stress mediated by protein kinase c and NADPH oxidase pathway (Bergamo et al., 2004).

Decreases endothelial cell proliferation induced by basic fibroblast growth factor (Moon et al., 2003), and serum and mammary gland VEGF (Ip et al., 2003), all of which are potent angiogenic factors expressed in many tumors including mammary gland.

Activates peroxisome proliferator-activated receptors (PPAR; Moya-Camarena et al., 1999; Belury, 2002a).

Activates transcription factors regulating gene expression with respect to cell growth, differentiation, and apoptosis (Majumdar et al., 2002).

Downregulates cyclooxygenase-2 activity related to carcinogenesis and inflammation (Watkins et al., 1999; Iwakiri et al., 2002).

Modulates arachidonic acid metabolism and reduces $PGE_2$ and cytokines (Banni et al., 1999; Urquhart et al., 2002; Iwakiri et al., 2002; Ochoa et al., 2004).

Decreases DNA synthesis associated with angiogenesis (Oh et al., 2003; Moon et al., 2003) and modulates DNA adduct formation (Majumdar et al., 2002).

Reduces nitric oxide and nitric oxide synthase (Iwakiri et al., 2002).

Increases retinal, which influences gene expression through activation of PPAR (Carta et al., 2002).

Reduces free radical induced oxidation (Belury, 2002a,b; Yu et al., 2002).

Blocks cell cycle in the mitotic division of cancer cells (Liu et al., 2002; Ochoa et al., 2004).

Downregulates expression of estrogen receptors bpth at mRNA and protein levels and decreases the binding activity of nuclear protein to estrogen (Tanmahasmut et al., 2004).

Inhibits IGF-I receptor signaling pathway implicated in the development of bladder cancer (Oh et al., 2003) or reduces IGF-II secretion (Cho et al., 2003b).

Downregulates ErbB2 and ErbB3 mRNA and protein levels (Cho et al., 2003a).

The lowest dose shown to be effective against mammary cancer in rats was 0.1% of the diet (Ip et al., 1999). Both free-fatty acid and triglyceride-CLA were essentially identical in their anticancer properties (Ip et al., 1995). This may have practical implications in human health, because the CLA present in foods from ruminants is mostly in triglyceride form and a mere intake of such CLA-enriched foods may help us derive the health benefits of CLA. At the 0.5% dose level, the anticancer activity of *c*-9, *t*-11 and *t*-10, *c*-12 CLA was very similar, even though accumulation of *t*-10, *c*-12 CLA in the mammary tissue was considerably less than that of *c*-9, *t*-11 CLA (Ip et al., 2002). The anticarcinogenic effects of both the isomers were greater at higher concentrations, >0.25% than at 0.1% of diet, with a proposition that the effects of the two isomers might not be additive (Hubbard et al., 2003). No adverse effect of a mixture of *c*-9, *t*-11 and *t*-10, *c*-12 CLA isomers in rats at 2,433 mg/kg bw for males and 2,728 mg/kg BW for females have been observed (O'Hagan and Menzel, 2003). It may have practical implications in human health in developing the drugs once the mechanism of action and the effective dose of CLA have been determined.

It was pointed out above about a possible hypothesis of TVA being converted to CLA in non-ruminants. It was based on the observations of higher concentrations of CLA in the mammary gland and other tissues for rats fed CLA-enriched butterfat than rats fed synthetic CLA isomers at the same dietary level (Ip et al., 1999). In rats fed TVA and *c*-9, *t*-11 CLA, conversion of dietary TVA to *c*-9, *t*-11 CLA resulted in a dose-dependent increase in the accumulation of *c*-9, *t*-11 CLA in the mammary fat pad, which was accompanied by a parallel decrease in tumor formation in the mammary gland (Corl et al., 2003). Similarly, after 4 d of incubation of SW480 and MCF-7 cancer cells with TVA at 5 and 20 microg/mL, *c*-9, *t*-11 CLA increased from undetectable levels to 8.57 and 12.14 g/100 g fatty acid methyl esters in cellular lipids, respectively (Miller et al., 2003a). These findings not only confirmed the conversion of TVA to *c*-9, *t*-11 CLA in non-ruminants, but also showed TVA to be equally important for cancer prevention as the dietary supply of *c*-9, *t*-11 CLA. Furthermore, it will also enhance the nutritional and therapeutic value of milk and meat from ruminants in preventing various forms of cancer.

**CLA and diabetes**

The role of CLA in regulating type-2 diabetes, which is linked to obesity, is not only complex and not so well understood, but also conflicting at times. Since *t*-10, *c*-12 CLA is linked to decreased body fat, it is this isomer that is implicated as an antidiabetic. It has been shown that CLA was as equally effective as thiazolidinediones, a class of oral insulin sensitizing agents that improve glucose utilization without stimulating insulin release, in reducing fasting glucose in Zucker diabetic rats (Belury and Vanden Huevel, 1999). In a double blind study with human diabetics, Belury (2002b) showed a decreased blood

**Table 3**. Biological effects of CLA on atherogenesis, adipogenesis, bone metabolism, and immune functions

| Study/model/cell line | Effects | Isomer* |
|---|---|---|
| A. On adipogenesis | | |
| Mice and rats[5] | Increased UCP2 expression in rats/no effect in mice | d |
| Type II diabetic subjects[6] | Reduced body weight and leptin | b |
| Male stD ddY mice[7] | Increased fat oxidation and $O_2$ consumption | c |
| Healthy, weight-stable women[8] | No effect on FA and glycerol metabolism | c |
| Healthy women[9] | Transient decrease in leptin, no effect on plasma glucose or lactate | c |
| ICR and C57BL/6J mice[10] | Reduced brown and white adipose tissue, | d |
| Balb-C mice[11] | Increased expenditure and loss of energy | c |
| Weaned piglets fed high-fat diet[12] | No effect on adipocity | c |
| Overweight and obese humans[13] | Reduced body fat mass | a/b |
| Abdominally obese men[14] | No change in body composition, or weight | b |
| Women (20–41 yr age)[15] | No effect on body composition and energy expenditure | c |
| B. On atherogenesis | | |
| Rabbits[1] | Reduced severity of lesions | a/b |
| Rabbits[2] | Reduced atherosclerosis in aortas | d |
| Rabbits[3] | Inhibited atherogenesis, established atherosclerosis regressed | c |
| Hamsters[4] | Reduced aortic fatty streak and total cholesterol | c |
| C. On immunity | | |
| Several[16] | Enhanced immune response with protection against collateral damage | c |
| Nursery pigs[17] | Enhanced lymphocyte proliferation | c |
| Young healthy women[18] | No change in immune status | c |
| D. On bone metabolism | | |
| Rats[19] | Reduced eicosanoid production | c |
| Rats[20] | Increased collagen synthesis | c |
| E. On oxidation | | |
| *In vitro*[21] | Protection from $H_2O_2$ or cumene $H_2O_2$ | a/b |
| Cell culture[22] | Quenched free radicals | a |
| *In vitro*[23] | Increased total oxyradical scavenging capacity | b |
| Rat liver[24] | Microsomes/mitochondria protected from $H_2O_2$ | d |

* a=*c*-9, *t*-11. b=*t*-10. *c*-12. a/b=*c*-9. *t*-11 and *t*-10. *c*-12. c=mixture. d=not known.
[1] Kritchevsky (2003). [2] Lee et al. (1994). [3] Kritchevsky et al. (2000). [4] Nicolisi et al. (1997). [5] Ealey et al. (2002). [6] Belury et al. (2002). [7] Ohnuki et al. (2001). [8] Zambell et al. (2001). [9] Medina et al. (2000). [10] Takahashi et al. (2002). [11] Terpstra et al. (2002). [12] Demaree et al. (2002). [13] Blankson et al. (2000). [14] Risérus et al. (2002). [15] Zambell et al. (2000). [16] Cook et al. (2003). [17] Bassaganya-Riera et al. (2001). [18] Kelley et al. (2000). [19] Li and Watkins (1998). [20] Watkins et al. (1999). [21] Su et al. (2003). [22] Yu et al. (2002). [23] Leung and Liu (2000). [24] Palacios et al. (2003).

glucose and plasma leptin in CLA supplemented patients. Belury et al. (2003) suggested that *t*-10. *c*-12 isomer may be the bioactive isomer of CLA that influences the body weight changes observed in subjects with type-2 diabetes. Although serum insulin was higher in CLA fed rats than thiazolidinediones treated rats, it was only half the amount observed in control rats (Belury and Vanden Huevel. 1999). Later, Henrickson et al. (2003) demonstrated that the improved glucose tolerance and insulin-stimulated glucose transport in the skeletal muscle of obese Zucker diabetic rats was due to *t*-10. *c*-12 isomer with no effect due to *c*-9, *t*-11 isomer. In contrast, *t*-10. *c*-12 isomer of CLA was shown to induce hyperinsulinemia and fatty liver in mice with no effect due to *c*-9. *t*-11 isomer (Clément et al., 2002). Similarly, *t*-10. *c*-12 isomer of CLA promoted insulin resistance, increased serum glucose and insulin concentrations. whereas *c*-9, *t*-11 isomer had ameliorative effect on lipid metabolism in *ob/ob* mice (Roche et al., 2002). Similarly, Brown et al. (2003) demonstrated that *t*-10, *c*-12 isomer of CLA decreased insulin-stimulated glucose uptake and metabolism in differentiating human preadipocytes. Some of the pertinent findings about the role of CLA in diabetes are presented in Table 2.

The initial proposition about the activation of PPARγ in modulating diabetes (Belury and Vanden Heuvel, 1999; Moya-Camarena et al., 1999) seems to be questioned with a new finding that showed a decreased expression of PPARγ by *t*-10. *c*-12 (but not *c*-9. *t*-11) in adipocytes. which could promote insulin resistance and oppose the hypoglycemic actions of thiazolidinediones *in vivo* (Brown and McIntosh. 2003; Brown et al., 2003). This could be the reason why Risérus et al. (2002) found an increased insulin resistance and glycemia in abdominally obese men when treated with 3.4 g/d of *t*-10. *c*-12 isomer of CLA. Some of the concerns about insulin resistance in humans (Risérus et al., 2002) and mice (Roche et al., 2002) as well as fatty liver associated with CLA (Clément et al., 2002) could probably be eliminated with arginine-CLA (Kim et al., 2004), because arginine infusion is known to have a preventive role in the insulin resistance by decreasing total plasma homocysteine

concentration (Cassone Faldetta et al., 2002). Put together, the effects of CLA on type-2 diabetes are not very clear and therefore further research on the effects, doses, and mechanisms of action of CLA is warranted.

**CLA and lipid metabolism**

Many of the effects of CLA mentioned above appear to stem from its ability to modulate lipid metabolism in animals, humans, and cell culture studies (Table 3). A major effect of CLA in this respect is the reduction of lipid uptake by adipocytes (Pariza et al., 2003), which leads to the reduction in body fat gain (Kim et al., 2002b). Both isomers of CLA were effective in reducing body fat in mice, with *c*-9, *t*-11 being more effective in females than in males (Chardigny et al., 2003). Feeding mice a diet with 0.5% *t*-10, *c*-12 CLA for 4 weeks reduced body fat gain, serum leptin levels, and adipocyte leptin mRNA expression without affecting feed intake or body weight (Kang and Pariza, 2001). Granlund et al. (2003) demonstrated that *t*-10, *c*-12 CLA prevented lipid accumulation in humans and mouse adipocytes at very low concentrations. Dietary CLA altered adipose tissue and milk lipids of pregnant and lactating sows and influenced the growth and tissue composition of weaned pigs (Bee, 2000a,b). Similarly, CLA decreased backfat, shoulder fat as well as carcass fat in pigs, with results more pronounced in females than in males (Dunshea et al., 2002). There was, however, no effect of CLA on adiposity of early-weaned piglets fed high fat diets (Demaree et al., 2002). In chicks, supplementation of CLA in the diet linearly increased its proportion in leg muscle (An et al., 2003), making it a potential source of CLA for humans. However, excessive dietary CLA also resulted in increased liver weight, hepatic lipid accumulation, and serum glutamic-oxaloacetic transaminase (An et al., 2003). Serum glutamic-oxaloacetic transaminase is particularly important, because it is the most sensitive indicator of tissue damage in avian species (Lumeij, 1997). In dairy cows, *t*-10, *c*-12 CLA reduces milk fat content, lipogenic rates, and expression of genes involved in milk lipid synthesis (Baumgard et al., 2001; 2002) and coordinates suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis (Peterson et al., 2003).

Azain et al. (2000) reported that the reduction in body fat mass in rats was the result of reduced adipose tissue cell size rather than cell number. Similarly, Poulos et al. (2001) found a reduction in cell size, but not the cell number, in rats that had less body fat in response to CLA supplementation. Such a decrease in body fat of mice fed CLA could also be due to an increase in energy expenditure (West et al., 2000; Terpstra et al., 2002) and energy loss in the excreta (Terpstra et al., 2002). Even a single dose of a mixture of CLA administered orally appeared to enhance energy metabolism in mice through increased fatty acid oxidation and sympathetic nervous activity (Ohnuki et al., 2001). Moreover, CLA affected the gene expression of proteins regulating energy metabolism in mice (Takahashi et al., 2002). Another way CLA reduces fat mass in mice is through apoptosis and lipodystrophy via tumor necrosis factor α and uncoupling protein 2 (Tsuboyama-Kasaoka et al., 2000). Apoptosis of mice adipose tissues occurred within 5 days of consuming a diet containing CLA (Miner et al., 2001). Evans et al. (2000) found the effects of *t*-10, *c*-12 isomer to be more pronounced than those of the crude mixture of CLA isomers. They further suggested that CLA's antiobesity effects were through inhibition of proliferation, increased FA oxidation, attenuating triglyceride content, and/or inducing apoptosis in preadipocytes. In another study, prevention of lipid accumulation in human and mouse preadipocytes by *t*-10, *c*-12 CLA was achieved through modulation of PPARγ (Granlund et al., 2003). Downregulation of $\Delta^9$-desaturase gene expression in adipocytes by *t*-10, *c*-12 isomer of CLA may contribute to the mechanisms by which CLA reduces body fat in mice (Choi et al., 2000). Such a loss of $\Delta^9$-desaturase function protects mice against adiposity and one of the consequences of $\Delta^9$-desaturase deficiency is an activation of lipid oxidation in addition to reduced triglyceride synthesis and storage (Ntambi et al., 2002). This is important because over expression of $\Delta^9$-desaturase is related to genetic predisposition to hepatocarcinogenesis in mice and rats (Falvella et al., 2002).

Determination of whether the body composition effects observed in animal models are applicable to humans is very important. Banni et al. (1999) suggested that the anticarcinogenic action of CLA stems from its metabolism and influence on tissue lipid metabolism, which CLA appears to modulate in a myriad of ways. In human subjects with type-2 diabetes, Belury et al. (2003) suggested that *t*-10, *c*-12 is the bioactive isomer that influences the body weight changes. In overweight and obese humans, CLA reduced body fat mass (Blankson et al., 2000). Smedman and Vessby (2001) showed a reduction in body fat in humans without affecting body weight when healthy men and women were supplemented with 4.2 g/d of a mixture of CLA containing equal amounts of *t*-10, *c*-12 and *c*-9, *t*-11 isomers. Noone et al. (2002) showed a significant improvement in fasting triglyceride and very low density lipoprotein metabolism in healthy human subjects when 3 g/d of a blend of *c*-9, *t*-11 and *t*-10, *c*-12 (50:50 or 80:20) CLA was supplemented in the diet. Lipogenesis was inhibited by *t*-10, *c*-12 but not *c*-9, *t*-11 isomer in primary cultures of stromal vascular cells from human adipose tissue (Brown et al., 2001). In contrast, a mixture of CLA had no significant effect on body composition, energy expenditure, fat oxidation, and respiratory exchange ratio at rest or during exercise in healthy adult women (Zambell et

al., 2000). Such differences on human adiposity may be the result of different mixtures and levels of CLA isomers and diverse subject populations (Evans et al., 2002).

To sum up, potential antiobesity mechanisms of CLA include decreased preadipocyte proliferation and differentiation into mature adipocytes, blocking of cell cycle during mitotic division, apoptosis of the adipocyte cells, decreased fatty acid and triglyceride synthesis, downregulation of key lipogenic enzymes, and increased energy expenditure, lipolysis, and fatty acid oxidation. Carta et al. (2002) went one step further and concluded that a regular intake of CLA and/or TVA as its precursor should work as an excellent preventive agent that would modulate lipid metabolism in target tissues thus conferring protection against different health problems associated with obesity. However, Loor et al. (2003) suggested that consumption of CLA supplements containing *t*-10, *c*-12 be avoided during nursing period because of its negative effects in body weights and carcass fat, protein, and ash contents.

**CLA and atherosclerosis**

Lee et al. (1994) showed initially that CLA has an antiatherosclerotic effect in rabbit aortas. There was a 34% reduction in atherosclerosis in rabbits when CLA was included at 0.1% of the diet for 12 weeks, which increased to 64% when included at 0.5% of the diet with a slight reduction to 58% at 1% of the diet (Lee et al., 1994). A significant reduction in total cholesterol, non-high density lipoprotein cholesterol, and aortic fatty streak areas in hamsters occurred even at 0.06% of CLA in the diet (Nicolisi et al., 1997). Inhibition of atherosclerosis and regression of established atherosclerosis (Kritchevsky et al., 2000) as well as a reduction in the severity of established lesions (Kritchevsky, 2003) in rabbits has been observed. Sher et al. (2003) showed a reduction in plasma cholesterol during cholesterol supplementation, but accentuation of the atherogenic lipid profile during acute phase response in hamsters when CLA was supplemented in the diet at 1%. Since a mixture of CLA isomers was used in most of these studies, the effects of specific isomers is not known. Some of the important findings about the effects of CLA on atherosclerosis are presented in Table 3.

Since it is difficult to study the effect of CLA on atherosclerosis in humans, an indirect approach by measuring various potential heart disease markers is required (Belury, 2002a). Lipid atherogenic risk markers were more favorably influenced by *c*-9, *t*-11 isomer than a mixture of CLA or fish oil (Valeille et al., 2004). When healthy human subjects were used in a double-blind placebo controlled intervention trial, Noone et al. (2002) demonstrated that a blend of *c*-9, *t*-11 and *t*-10, *c*-12 isomers (80:20 and 50:50) improved very low-density lipoprotein cholesterol and plasma triacylglycerol metabolism suggesting that some of the cardio-protective effects of CLA shown in animal studies were relevant to humans as well. However, there was no effect of supplementing CLA on total cholesterol or high-density lipoprotein cholesterol in healthy human subjects (Mougios et al., 2001). In another study in humans, supplementation of CLA did not affect any of the atherogenic parameters (Benito et al., 2001). In totality, these results indicate that antiatherogenic properties of CLA are not definitive.

**CLA and immune system**

Enhanced immune function is usually associated with anorexia and wasting. In a detailed review, Cook et al. (2003) showed that CLA not only enhances immune response, but also protects tissues from collateral damage (Table 3). Sugano et al. (1999) proposed that the immune enhancing effect of CLA was by modulating eicosanoid and immunoglobulin production. Whigham et al. (2000) concluded that *t*-10, *c*-12 isomer competitively inhibited the conversion of arachidonic acid to prostaglandin $E_2$. CLA also diminished lipopolysaccharide-induced inflammatory events in macrophages through reduced mRNA and protein expression of nitric oxide synthase and cyclooxigenase-2 as well as subsequent production of nitric oxide and prostaglandin $E_2$ (Cheng et al., 2004), both of which are also implicated in carcinogenesis. Cook et al. (1999) suggested that CLA prevents immune associated wasting by protecting nonlymphoid tissues from the adverse effects of cytokines, which are growth suppressants, because CLA influences the immune system by altering the effects of cytokine, interleukin, leukotriene and many immunoglobulins (Sébédio et al., 2000). Although dietary CLA enhanced antibody production in broiler chickens (Takahashi et al., 2003), ameliorated viral infectivity in a pig model of virally induced immunosuppression (Bassaganaya-Riera et al., 2003), and enhanced lymphocyte proliferation in nursery pigs (Bassaganaya-Riera et al., 2001), no change in immune status was observed in young healthy women (Kelley et al., 2000). Whigham et al. (2002) indicated that CLA might induce a change in immune response in favor of cell-mediated response rather than an allergic one, while Nichenametla et al. (2004) showed a higher natural killer cell cytotoxicity in rats fed CLA in diet than the control group without CLA. It appears that *c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA stimulate different immunological events in mice with *c*-9, *t*-11 increasing tumor necrosis factor α while *t*-10, *c*-12 increasing immunoglobulin A and M production (Yamasaki et al., 2003). In a double blind parallel reference-controlled intervention study in adult humans, almost twice as many subjects reached protective antibody levels to hepatitis B when consuming a 50:50 mixture of *c*-9, *t*-11 and *t*-10, *c*-12 CLA isomers for 12 weeks compared with sunflower oil fed

reference subjects, but the response to 80:20 mixture of *c*-9, *t*-11 and *t*-10, *c*-12 CLA was similar to that of reference (Albers et al., 2003). This is probably the first of its kind about the effects of CLA on immune function using actual human subjects and may open new vistas for CLA research. The implications of immunomodulatory effects of CLA on livestock production would probably be in enhancing the response of animals to vaccination and conferring disease resistance. To date there have been limited attempts at identifying the effects of specific isomers of CLA on the immune system.

**CLA and bone metabolism**

Watkins et al. (1996) found a higher rate of bone formation in chicks fed butterfat, which was suggested to be due probably to increased CLA intake. Dietary CLA led to differences in CLA enrichment of various organs and tissues, bone marrow and periosteum containing the highest concentrations of CLA and brain the lowest (Li and Watkins, 1998). Enrichment of chondrocytes with CLA affected collagen synthesis in a dose dependent fashion (Watkins et al., 1999). Reduced production of arachidonic acid and $PGE_2$ in the chondrocytes was suggested to be the possible mechanism (Watkins and Siefert, 2000). Such changes in bone biomarkers and bone formation rates in rats were associated with increased *c*-9, *t*-11 CLA in bone tissue lipids (Watkins et al., 2003). Furthermore, dietary beef fat and a CLA supplement were able to maintain synthetic activity of osteoblastic cells and CLA was even able to rescue the reduced bone formation rate in rats given a diet high in ω-6 FA (Watkins et al., 2003). McDonald (2000) suggested increased ash content in CLA fed animals (Park et al., 1999) is due to protection of bone loss from cytokines. Further investigation is needed as to how bone metabolism is affected by CLA and mechanism of action related with it.

**CLA and oxidation**

Some of the potential health benefits of CLA appear to be mediated through its antioxidant properties (Table 3). Yu et al. (2002) showed that both *c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA quench free radicals. It was also shown that the mixture of both isomers was more effective than either isomer alone. When the effect of CLA on paraoxygenase 1, one of the antioxidant proteins associated with high density lipoproteins, was studied in vitro, both *c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA showed 71 to 74% protection of paraoxygenase 1 (Su et al., 2003). Additionally the two isomers also protected paraoxygenase 1 from oxidative inactivation of $H_2O_2$ or cumene hydroperoxide (Su et al., 2003). The *c*-9, *t*-11 isomer of CLA scavenged more free radicals at steady state (Yu et al., 2002) and was more effective in protecting paraoxygenase 1 than *t*-10, *c*-12 isomer (Su et al., 2003). However, Leung and Liu (2000) reported a stronger oxyradical scavenging capacity for *t*-10, *c*-12 than *c*-9, *t*-11 isomer. In rat liver, CLA was more effective than vitamin A in protecting microsomes or mitochondria from peroxidative damage (Palacios et al., 2003). These results indicate that both *t*-10, *c*-12 and *c*-9, *t*-11 isomers of CLA are effective antioxidants. Kim et al. (2004) investigated the antioxidant activities of arginine-CLA, a water-soluble salt. They found free radical scavenging capacity of arginine-CLA was double that of CLA with its antioxidant activity similar to vitamin E. Arginine-CLA may have further implications in expanding the scope of the application of CLA as a health-promoting agent because of its solubility in water.

## IMPLICATIONS

Diverse biological roles of CLA in mediating cancer, diabetes, lipid metabolism, atherosclerosis, immune function, bone modeling etc. observed in animal models are quite compelling. Although there have been a few attempts at verifying the positive effects of CLA in human health through case control studies, it is not yet possible to clearly state that CLA supports all those benefits in humans as well. Limited available literature in humans, however, also supports the findings observed in animals and tissue culture models even though the results are inconclusive and even conflicting at times. Mechanisms of action with respect to each isomer or a mixture of CLA isomers vis-à-vis its physiological roles remains speculative, which demands further research and thoughtful insight to put together the bits and pieces associated with the changes brought about by CLA. Dose related studies, both in animals and in humans, are very limited. In spite of so much research efforts, testing CLA in clinical trials is still remote. Further experimentation is warranted in characterizing the dose-response profile of each or a mixture of CLA isomers with each of the physiological role associated with it, both under in vivo and in vitro conditions. As a result, natural enrichment of food products through manipulation of animal diet may contribute to the overall goal of obtaining the positive health benefits associated with CLA in the immediate future.

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