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AN UPDATE ON THE BENEFICIAL ROLES OF CONJUGATED LINOLEIC ACID (CLA) IN MODULATING HUMAN HEALTH: MECHANISMS OF ACTION – A REVIEW

Sa'eed Bawa

Section of Dietetics, Department of Dietetics and Functional Foods, Faculty of Human Nutrition and Consumer Sciences, Warsaw Agricultural University, Warsaw

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Conjugated linoleic acid (CLA) as a derivative of linoleic acid (LA) is a generic term used to refer to a mixture of geometrical and positional isomers in which up to 16 members have been identified. CLA is unique because unlike most antioxidants which are components of plant products, it is found in foods of animal origin, such as dairy products and meats. CLA concentrations in dairy foods range from 2.9 to 8.92 mg/g fat of which the 9-*cis*, 11-*trans* isomer makes up from 73% to 93% of the total CLA. Many potentially beneficial health effects have been ascribed to these fatty acids when consumed as a mixture, where generally the 9-*cis*, 11-*trans* isomer (rumenic acid) and the 10-*trans*, 12-*cis* isomer dominate. Anti-obesity, anticarcinogenic, anti-diabetic, anti-atherosclerotic, and immune modulation are among the most spectacular health benefits of CLA. The aim of this review was to show the pleiotropic biological activity of this compound (depicted in the literature recently), with particular references to its mechanisms of action.

INTRODUCTION

Diet is considered a contributing factor to the development of the so-called diseases of modern civilization, such as cancer, atherosclerosis, obesity, osteoporosis, type 2 diabetes mellitus, and insulin resistance. A few compounds in the human diet have been identified and shown to possess anti-atherosclerotic, anti-carcinogenic and anti-obesity properties, but most of them are of plant origin and are only found in small concentrations. However, conjugated linoleic acid (CLA), a component of ruminant fat continues to intrigue scientists from different work of life in terms of its role in delaying or inhibiting the onset of diet-related diseases [Turini & Martin, 2001; Belury, 2002]. Conjugated linoleic acid refers to a mixture of positional and geometric isomers of linoleic acid (18 carbons) with two double bonds separated by one single bond.

Unlike essential fatty acids where the double bonds are methylene-interrupted, CLA isomers are consecutive, that is, conjugated. Furthermore, each double bond can be in the *cis* or *trans* configuration. Therefore, many forms of CLA are possible [Rickert *et al.*, 1999; Yurawecz *et al.*, 1999a, b], but the main form found in foods from ruminant animals is the *cis*-9, *trans*-11 CLA, known trivially as rumenic acid. CLA concentrations in dairy foods range from 2.9 to 8.92 mg/g fat of which the 9-*cis*, 11-*trans* isomer makes up from 73% to 93% of the total CLA. Many potentially beneficial health effects have been ascribed to these fatty acids when consumed as a mixture, where generally the 9-*cis*, 11-*trans* isomer and the 10-*trans*, 12-*cis* isomer dominate. Anti-obesity, anticarcinogenic, anti-diabetic, anti-atherosclerotic, and immune modulation are among the most spectacular health benefits of CLA. This review will address the potential health benefits of CLA in humans as well as pleiotropic biological activity of this compound (depicted in the literature recently), with particular references to its mechanisms of action.

THE ORIGIN OF CLA

BIOCHEMISTRY

Conjugated linoleic acid (CLA) is a term used to describe a group of positional and geometric isomers of linoleic acid (18:2 n-6 or 9,12-*cis*, *cis*-octadecadienoic acid, LA), in which the double bonds are conjugated, instead of being in the typical methylene-interrupted configuration [Decker, 1995]. The conjugated double bonds occur at carbon atoms 10 and 12 or 12 or 9 and 11, with all possible *cis* and *trans* combinations. Although conjugation of double bonds takes place as part of free radical-mediated oxidation of LA, CLA is a true isomer of LA, because it does not possess additional oxygen [Van den Berg *et al.*, 1995]

CH_3-$(CH_2)_5$-CH=CH=CH=CH-$(CH_2)_7$-COOH 9*c*,11*t*-18:2.
trans *cis*

DIETARY SOURCES OF CLA AND ITS METABOLISM

There are many isomers of CLA (nine different positional and geometric isomers have been reported in minute components of food products), and the distribution of

Author's address for correspondence: Sa'eed Bawa, Department of Dietetics & Functional Foods, Faculty of Human Nutrition & Consumer Sciences, Warsaw Agricultural University, ul. Nowoursynowska 159C, 02-776 Warsaw, tel/fax: (48 22) 847 37 45; e-mail: halilu@alpha.sggw.waw.pl

naturally occurring isomers differs significantly from that commercially produced [Sahet *et al.*, 1999; Yurawecz *et al.*, 1999 a, b]. Food products derived from ruminant animals are the major source of CLA in human diets [McGuire & McGuire, 1999]. Fat of ruminant origin (beef, lamb and dairy) contains much higher concentrations of CLA than fat from non-ruminants. CLA levels in dairy products normally range from 2.9 to 8.92 mg/g fat, of which the 9-*cis*, 11-*trans* isomer comprises 73% to 93% of the total CLA. Beef also contains CLA in a similar range with the 9-*cis*, 11-*trans* isomer contributing from 57% to 85% of total CLA. Vegetable oils and margarines contain little CLA. The amount of CLA in vegetable oils and fats originating from animals, other than ruminants, typically ranges from 0.6 to 0.9 mg/g fat. Physiological concentrations of CLA from different foods are given in Table 1.

Although different CLA isomers are found in foods for humans, traditionally the predominant form has been rumenic acid (RA). RA represents more than 80% of the CLA present in milk fat and over 75% of the CLA found in beef fat [Sahet *et al.*, 1999; Yurawecz *et al.*, 1999a].

CLA occurring in milk and meat fat of ruminants originates from two sources [Griinari & Bauman, 1999]. One source is CLA synthesized during the process of biohydrogenation of linoleic acid in rumens. The second source is CLA formed in the animal's tissues from *trans*-11 $C_{18:1}$, an intermediate in the biohydrogenation of unsaturated fatty acids. Therefore, the uniqueness of CLA in food products of ruminant origin relates to the incomplete biohydrogenation of dietary unsaturated fatty acids.

Ironically, rumen biohydrogenation of dietary lipids is responsible for the high levels of saturated fatty acids in fat of ruminants, a feature considered as undesirable for some aspects of human health.

When consumed by ruminant animals, dietary fats undergo two important biotransformations in the rumen.

TABLE 1. Conjugated linoleic acid content of various foods [MacDonald, 2000].

Product	[mg/g fat]	Product	[mg/g fat]
Dairy product		Meat/fish	
Homogenized milk	5.5	Fresh ground beef	4.3
2% milk	4.1	Veal	2.7
Butter fat	6.1	Lamb	5.8
Condensed milk	7.0	Pork	0.6
Cultured buttermilk	5.4	Chicken	0.9
Butter	4.7	Fresh ground turkey	2.6
Sour cream	4.6	Salmon	0.3
Ice-cream	3.6	Egg yolk	0.6
Low-fat yoghurt	4.4	Vegetable oils	
Custard-style yoghurt	4.8	Safflower oil	0.7
Plain yoghurt	4.8	Sunflower oil	0.4
Frozen yoghurt	2.8		
Medium cheddar	4.1		
American processed	5.0		

The initial biotransformation is degradation of the ester linkages catalyzed by microbial lipases. This step is a prerequisite for the second biotransformation, that is, biohydrogenation of the unsaturated fatty acids. Bacteria are mainly responsible for bohydrogenation of unsaturated fatty acids in the rumen, but protozoa seem to be of minor importance. Until recently, the only bacterium known to be capable of biohydrogenation was *Butyrivivrio fibrisolvens*, but recent researches show that a diverse range of rumen bacteria have the capacity to biohydrogenate unsaturated fatty acids [Griinari & Bauman, 1999].

The biohydrogenation sequence of linoleic acid is presented in Figure 1.

Isomerization of the *cis*-12 double bond represents the initial step during biohydrogenation of fatty acids

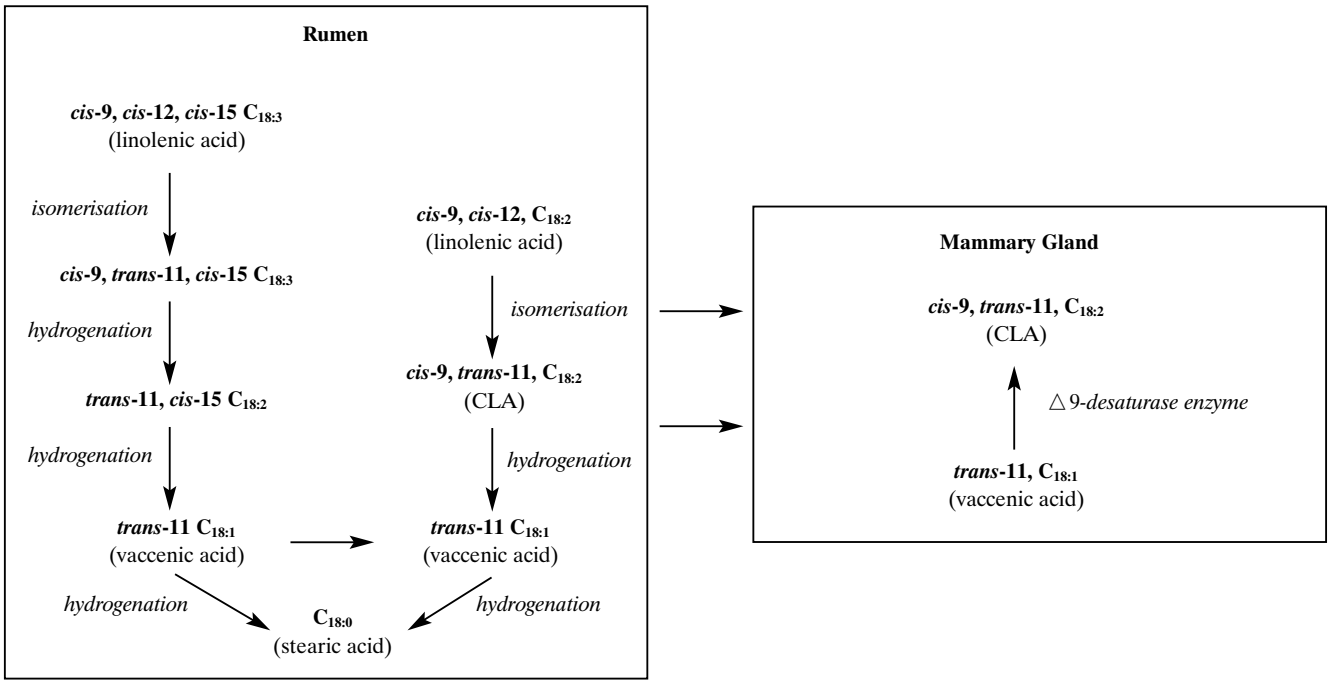

FIGURE 1. Production of CLA on the dairy cow and its metabolism.
Two sources: (1) Incomplete bacterial hydrogenation of linoleic acid in the rumen; (2) Endogenous synthesis in the mammary gland from vaccenic acid [Griinari & Bauman, 1999].

containing a *cis*-9, *cis*-12 double bond system. The isomerase reaction is unusual, because it does not require any cofactors and occurs in the middle of a long hydrocarbon chain, remote from any activating functional groups. Linoleate isomerase (EC 5.2.1.5) is the enzyme responsible for the synthesis of conjugated double bonds from the *cis*-9, *cis*-12 double bond structure of linoleic acid as well as α- and γ-linolenic acids. This enzyme is bound to the bacterial cell membrane and demonstrates an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group.

The second reaction is a reduction, in which *cis*-9, *trans*-11 CLA is converted to *trans*-11 $C_{18:1}$. Isomerization of the *cis*-12 double bond is followed by rapid conversion of the *cis*-9, *trans*-11 CLA to *trans*-11 octadecenoic acid.

Hydrogenation of the *trans*-11 monoene occurs less rapidly, thus increases in concentration. Therefore, *trans*-11 $C_{18:1}$ reduction seems to be rate-limiting in the biohydrogenation sequence of unsaturated fatty acids. Consequently, this penultimate biohydrogenation intermediate accumulates in the rumen and hence becomes more available for absorption.

Similar to biohydrogenation of linoleic acid, biohydrogenation of linolenic acid begins with an isomerization followed by a sequence of reductions and terminates with the formation of stearic acid. The predominant $C_{18:3}$ fatty acid in forages and feedstuffs is α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 octadecatrienoic acid). Rumen biohydrogenation of α-linolenic acid yields *cis*-9, *trans*-11, *cis*-15 conjugated octadecatrienoic acid as the main initial isomerization product. This process is followed by reduction of the *cis*-double bonds. As a result of this, *trans*-11 octadecenoic acid is a common intermediate in the biohydrogenation of both linoleic acid and α-linolenic acid. Furthermore, biohydrogenation of γ-linolenic acid, *cis*-6, *cis*-9, *cis*-12 octadecatrienoic acid, also leads to the formation of *trans*-11 $C_{18:1}$ [Griinari & Bauman, 1999].

INHIBITION OF CARCINOGENESIS BY CLA

The National Academy of Sciences [NRC, 1996] publication *Carcinogenesis and Anticarcinogens in the Human Diet* concluded that "… conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals."

In several animal studies, supplementation of experimental diets with CLA has been shown to exert anticarcinogenic effect against chemically-induced cancers of the skin, forestomach, colon and breast [Ha *et al.*, 1990; Ip *et al.*, 1994; Belury *et al.*, 1996; Xu & Dashwood, 1999; Chen *et al.*, 2003]. Of the individual isomers of CLA, *c*9,*t*11 isomer has been shown to be the most active biologically since it is the predominant isomer incorporated into the phospholipids of cell membrane. However, recent research provided evidence that the *t*10,*c*12-CLA isomer might also possess biological activity [Sěbědio *et al.*, 1999]. Till date, all experimental diets as well as cell culture studies involved the use of a mixture of CLA isomers that contained mainly *c*9,*t*11-, *t*10,*c*12-, *t*9,*t*11-, *t*10,*t*12. Studies carried out by Ha *et al.* [1990], Ip *et al.* [1991], Schut *et al.* [1997], and Hubbard *et al.* [2000] demonstrated that potent anticarcinogenic effects were attributed to a synthetic mixture of conjugated linoleic acid composing mainly of *c*9,*t*11-, and *t*9*c*11-CLA (43%) and *t*10,*c*12-CLA (45.3%). The compound used in the experiment by Ha *et al.* [1990] contained *c*9,*t*11-, *t*10,*c*12-, *t*9,*t*11-, *t*10,*t*12-CLA, which accounted for about 90% of the material. Hubbard *et al.* [2000] applied a mixture of CLA isomers with 32.5% *c*9,*t*11-CLA and 32.5% *t*10,*c*12-CLA isomers making up 66% to mammary tumour matastasis. So far, no study has been conducted on the effects of CLA monomer on carcinogenesis in animal model.

Studies show that anticarcinogenic activities of CLA involve all the stages of carcinogenesis, including initiation [Josyula *et al.*, 1998a; b], post-initiation or promotion [Ip *et al.*, 1997; Kimoto *et al.*, 2001], progression [Ip *et al.*, 1995], and matastasis [Hubbard *et al.*, 2000; Xue *et al.*, 2001; Chen *et al.*, 2001; Cesano *et al.*, 1998].

The exact mechanisms, through which CLA modulates tumourigenesis are still debatable. Ha *et al.* [1990] suggested an antioxidant mechanism, which could be attributed to the decrease in linoleic metabolites (in particular arachidonic acid) in mammary gland tissue as the CLA intake increased [Banni *et al.*, 1999]. The accumulation of CLA and its metabolites in tissues may attenuate lipid peroxidation in these tissues by decreasing the concentration of linoleic acid-derived arachidonic acid [Livisay *et al.*, 2000] which is more prone to lipid oxidation and formation of mutagenic and genotoxic malondialdehyde than CLA. Inhibition of oxidative stress *in vitro* [Ha *et al.*, 1990] and *in vivo* [Ip *et al.*, 1991] indicates that CLA is a potent antioxidant in tissues. Studies by Ha *et al.* [1990] showed that compared with other antioxidants, CLA was more potent than α-tocopherol and as effective as butylated hydroxytoluene (BHT) in inhibiting iron-thiocyanate-induced peroxide formation. In addition, CLA was found to be as effective as vitamin E and butylated hydroxyanisole in inhibiting the formation of thiobarbituric acid reactive substances (TBARS), a biomarker frequently used to assess oxidation in biological systems in the mammary gland [Ip *et al.*, 1991].

However, some studies have shown that CLA possesses pro-oxidant effects rather than antioxidant. Chen *et al.* [1997] demonstrated a pro-oxidant activity of CLA in a dose-dependent manner in an experiment in which the oxygen uptake by canola oil (rich in polyunsaturated fatty acids which are prone to autooxidation) was monitored after heating to 90°C in the presence of CLA (0.1–1.0%). Van den Berg *et al.* [1995] compared the antioxidant activities of CLA, vitamin E and butylated hydroxytoluene (BHT) using unsaturated phospholipids model membranes that were exposed to a steady stream of oxyradicals. Oxidation of 1-palmitotoyl,2-linoleoyl phosphatidylcholine led to a steady increase in absorbance at 233 nm. In the presence of BHT, production of conjugated dienes was almost completely inhibited during a lag phase. BHT appeared more effective than vitamin E in suppressing the oxidation of 1-palmitotoyl,2-linoleoyl phosphatidylcholine. However, CLA did not induce a lag phase in oxidation. After 60 min of oxidative stress in the presence of CLA, peroxidation was only minimally lower than in the absence of CLA. Other experiments with the application of even more relevant *in vivo* oxidant model involving reactive oxygen species, such as H_2O_2 and pro-oxidant Fe^{2+}, showed that vitamin E always exhibited strong antioxidant activity, but no unequivocal antioxidant effect has ever been found for CLA [Van den Berg *et al.*, 1995].

Therefore, it has been postulated that CLA anticancer activity might be related to its induction of cytotoxic lipid peroxidation products, such as conjugated diene hydroperoxides. O'Shea *et al.* [1999; 2000] demonstrated that CLA is incorporated directly into cell membrane phospholipids and that, similar to the synthetic mixture of CLA isomers, CLA-enriched milk fat induced lipid peroxidation in MCF-7 cells and have suggested that conjugated diene hydroperoxides might generate an internal cellular pro--oxidant milieu that precedes the inhibition of growth-regulatory signals. This suggests that CLA may act at the level of gene expression in producing its cytotoxic action on tumour cells. CLA has been shown to increase the activity of three antioxidant defence enzymes, suggesting that this compound causes an imbalance in the pro-oxidant/antioxidant system in cells [O'Shea *et al.*, 1999, 2000]. Belury [1995] postulated that due to the conjugated structure of CLA, there is more efficient trapping of electrons in its double bond than in methylene-interrupted double bonds, and that antioxidant enzymes are induced as an adaptation to oxidant stress. *trans*-10, *cis*-12 CLA supplementation has recently been shown to increase oxidative and inflammatory biomarkers in obese men. Supplementation with this CLA isomer markedly elevated the level of 8-iso-$PGF_{2\alpha}$ (578%) and C-reactive protein (110%), markers of oxidative stress compared with placebo ($p<0.0001$ and $p<0.01$, respectively). The increase in 8-iso-$PGF_{2\alpha}$, but not in C-reactive protein, was significantly and independently related to aggravated insulin resistance.

These unfavourable effects of *trans*-10, *cis*-12 CLA might be of clinical importance with regard to cardiovascular disease, in consideration of the widespread use of dietary supplements containing this fatty acid.

It is worth underlying that cytotoxic compounds do not limit their activity only to tumor cells, so supplementation of diet with only CLA for the purpose of the prevention or treatment of malignant cells might be counterproductive.

The inhibition of carcinogenesis by CLA may result from its effects on prostaglandin synthesis. Due to structural similarities to linoleic acid, CLA isomers have been shown to undergo elongation and desaturation processes in different animal species and also in humans [Sěbědio *et al.*, 1999; 1997], retaining the conjugated diene structure. As a polyunsaturated fatty acid, from which 20 carbon atoms metabolites are produced, CLA metabolism may interfere with eicosanoid formation by different pathways, including (a) decrease in arachidonic acid supply, (b) inferring with lipoxygenase and cyclooxygenase pathways, and (c) formation of eicosanoid-like molecules which may compete with classically known eicosanoids. A number of studies indicate that CLA affects the synthesis of PGE_2, one of the prostaglandins that enhance carcinogenic processes [Li & Watkins, 1998]. Therefore, CLA seems to disturb linoleic acid metabolism and consequently the deposition of arachidonate and its metabolites, particularly in those tissues where CLA and some of its metabolites, such as conjugated 18:3 and conjugated 20:3 acid are preferentially incorporated. These tissues, include the adipose and mammary tissue in which CLA is incorporated into neutral lipid. Furthermore, conjugated 20:4 is preferably incorporated into specific phospholipids, primarily phosphatidylinositol and phosphatidylserine.

In adipose and mammary tissue, the metabolites content ranges from 5 to 15% of total CLA, and in plasma and liver from 10 to 30%. Other metabolites with 16 carbon atoms (conjugated 16:2 and 16:3 acids) arising most likely from peroxisomal beta-oxidation of CLA and its metabolites respectively, have been detected.

Another mechanism, through which CLA modulates carcinogenesis, may be inhibition of angiogenesis. Masso--Welch *et al.* [2002] showed that dietary CLA decreased serum levels of vascular endothelial growth factor (VEGF) and whole mammary gland levels of VEGF and its receptor Flk-1. Study by this group demonstrated that both *c*9,*t*11 and *t*10,*c*12-CLA isomers were effective in inhibiting angiogenesis *in vitro* in a dose-dependent fashion. Therefore, the ability of CLA to inhibit angiogenesis may contribute to its efficacy as a chemopreventive agent (Figure 2).

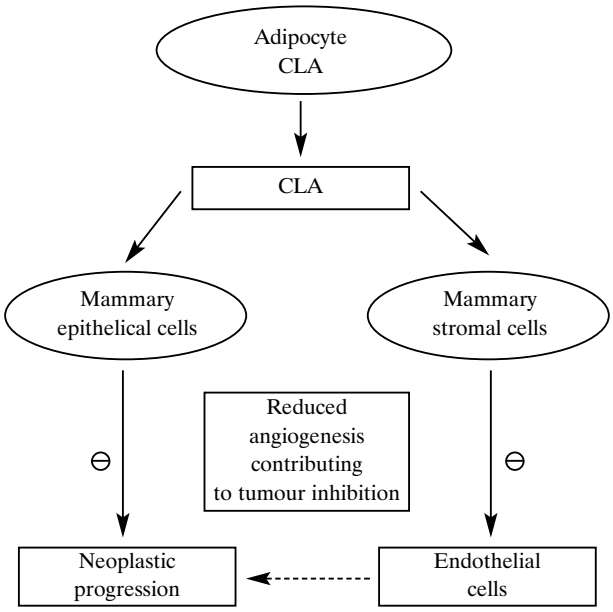

FIGURE 2. CLA as a chemopreventive agent – inhibition of angiogenesis; ⊖ inhibition.

It has been shown that CLA may exert its anticancer effect through the stimulation of apoptosis. Kim *et al.* [2002] found that *trans*-10, *cis*-12 CLA induced apoptosis and decreased DNA synthesis, whereas *cis*-9, *trans*-11 CLA had no effect in Caco-2 colon cell growth arrest. Kim *et al.* [2002] concluded that CLA-inhibited Caco-2 cell growth was mediated through a decrease in insulin-like growth factor-II (IGF-II) secretion in these cells. In most recent studies by Cho *et al.* [2003] it was shown that CLA enhanced apoptosis in HT-29 cells, which was probably mediated by its capacity to downregulate ErbB3 signalling and the phosphoinositide 3-kinase (PI3K/Akt) pathway. Activation of ErbB2 and ERbB3 has been implicated in the development of colon cancer and both proteins are expressed at high levels in the HT-29 cell line. Activation of ErbB2/ErbB3 heterodimers is regulated by the ErbB3 ligand heregulin. The studies by Cho *et al.* [2003] showed that CLA inhibited DNA synthesis and induced apoptosis of HT-cells and although the addition of heregulin-α led to increase in cell number, it was unable to counteract the negative growth regulatory effect of CLA. Furthermore, these studies revealed that CLA inhibited heregulin-α-

-stimulated phosphorylation of ErbB2 and ErbB3, recruitment of the p85 subunit of phosphoinositide 3-kinase to the ErbB3 receptor, ErbB3-associated PI3K activities, and phosphorylation of Akt. CLA decreased ErbB2 and ErbB3 mRNA and protein levels in a dose-dependent manner.

Despite numerous studies on the role of CLA in preventing breast cancer in animals, there is scarcity of epidemiological data on the anticancer activities of this compound in humans. In a recent study by Voorrips *et al.* [2002] evaluating the relationship between the intakes of CLA and other fatty acids and breast cancer incidence it was found that CLA intake showed a weak, positive relation with breast cancer incidence. However, significant inverse associations were found with monounsaturated and *cis* unsaturated fatty acids, whereas total fat and energy intake of CLA-containing food groups were not related to breast cancer incidence. It is worth mentioning that this study involved a 6.3 year of follow-up and 941 incident cases of breast cancer, multivariate rate ratios and 95% *cis* were computed for energy-adjusted intakes of fatty acids and CLA-containing food groups, for example, butter, cheese, milk, other milk products, and meat. Therefore, the anticarcinogenic mechanisms of the action of CLA in humans still remain to be elucidated.

NUTRIENT PARTITIONING AND MANAGEMENT OF FAT BODY MASS BY CLA ISOMERS

CLA has been shown to reduce body fat accumulation in several animal models. DeLany & West [2000] carried out studies in AKR/J mice and found that CLA reduces body fat accumulation whether animals are fed a high-fat or low--fat diet with no effect on food intake. In one of the most recent studies conducted in hamsters by Sher *et al.* [2003] it was found that CLA decreased body weight gain and adipose tissue reserves.

One mechanism by which CLA decreases body fat is an increase in energy expenditure, which is observed within 1 week of CLA feeding and is maintained for at least 6 weeks. The increased energy expenditure is sufficient to account for the decreased fat accumulation. DeLany & West [2000] found an increase in fat oxidation without a decrease in *de novo* fat biosynthesis with CLA feeding. CLA might affect energy metabolism by directly stimulating lipolysis and decreasing lipoprotein lipase. This action of CLA could lead to an increase in the needs for recycling between fatty acids and triacylglycerols or could direct fatty acids into beta-oxidation if storage was impaired. The later explanation has been confirmed by Park *et al.* [1997], who found an increase in the activity of carnitine palmityl transferase in muscle of animals treated with CLA. Findings by DeLany & West [2000] that CLA blocks the normal diurnal differences in respiratory quotient (RQ) is in concert with CLA stimulating lipolysis during the night, which leads to lowering the RQ. A recent experiment conducted by Terpstra *et al.* [2002] in young, growing, 5-week-old Balb-C mice showed that CLA-treated mice had a net loss of body energy which was accounted for by an increase in energy expenditure (74%) and by an increase in energy lost in the excreta (26%). However, feeding CLA also increased liver weight, which may warrant further studies on the safety of CLA, which is an unwanted phenomenon. Earlier studies with the use of laboratory animals by West *et al.* [1998], Park *et al.* [1999a, b], and Yamasaki *et al.* [1999] as well as studies in pigs conducted by Ostrowska *et al.* [1999] and Muller *et al.* [1999] showed that CLA isomers reduced body fat with the simultaneous increase in fat-free mass (FFM). Results of studies by Henrietta-Blankson *et al.* [2000] in overweight and obese humans clearly showed beneficial effects of CLA (in a dose of 3.4 g/d for 12 weeks) with regard to the reduction of body fat mass and an increase in lean body mass. However, due to the relatively small number of participants, a general conclusion on the effects of CLA isomers in overweight and obese humans cannot be drawn. The anti-obesity actions of CLA isomers (*trans*-10, *cis*-12) were demonstrated by Brown *et al.* [2001], who conducted studies in primary cultures of stromal vascular cells from human adipose tissue. *Trans*-10, *cis*-12 attenuated lipogenesis in these cells, but *cis*-9, *trans*-11 CLA increased the triacylglycerol content of the cultures.

Apoptosis in adipocytes and lipodystrophy is another mechanism by which CLA causes a decline in fat mass. This was shown in a study conducted by Tsuboyama-Kasaoka *et al.* [2000], who found that a dietary component brings about lipodystrophy and suggested that some agents that decrease fat mass may lead to lipodystrophy. Furthermore, Tsuboyama-Kasaoka *et al.* [2000] showed that a fat mass decline from CLA supplementation was due to apoptosis and that leptin treatment can reverse CLA-induced lipodystrophy. The apoptosis in adipocytes by CLA induced a marked increase in tumor necrosis factor-alpha (TNF-α) and uncoupling protein-2 (UCP2) which may account for the phenotypic features of lipodystrophy, such as a decrease in white and brown adipose tissue mass, lipid accumulation in the liver, and insulin resistance (Figure 3).

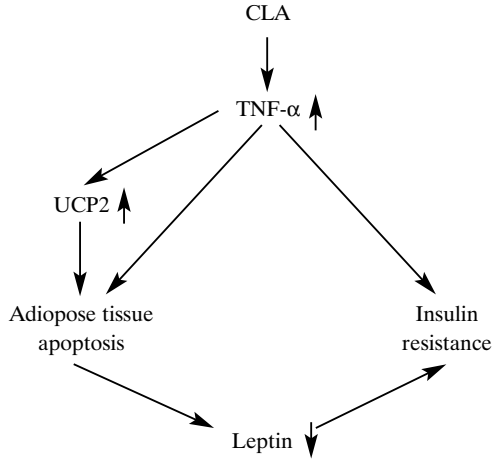

FIGURE 3. Possible mechanism of CLA-induced insulin resistance [Tsuboyama-Kasaoka *et al*., 2000].

Since CLA supplementation induces insulin resistance as a result of (TNF-α) and (UCP2) upregulation, then such a dietary approach cannot be recommended, because if uncontrolled, this could lead to non-insulin diabetes mellitus, obesity and other disorders related to this latter disease.

Azain *et al.* [2000] and Xu *et al.* [2003] demonstrated that the reduction in adipose tissue mass in response to dietary CLA in rats and mouse, respectively, is accounted for by a decrease in cell size rather than a change in cell

number. In a recent study by Belury *et al.* [2003] with the participation of patients with type 2 diabetes mellitus it was shown that plasma levels of CLA were inversely associated with body weight ($p < 0.05$). When levels of plasma *trans*-10 and *cis*-12-CLA isomer were correlated with changes in body weight or serum leptin, *trans*-10 and *cis*-12-CLA isomer, but not *cis*-9 and *trans*-11 CLA, was inversely related to body weight ($p < 0.05$) and serum leptin ($p < 0.02$). These studies strongly suggest that the *trans*-10 and *cis*-12--CLA isomer may be the bioactive isomer of CLA, which influences body weight changes seen in subjects with type 2 diabetes mellitus.

Some of the metabolic studies with the use of dietary CLA demonstrated clear differences between the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers on lipid metabolism. Studies in mice by Park *et al.* [1999a] showed that changes in body composition were attributed to the *trans*-10, *cis*-12 CLA isomers but not to *cis*-9, *trans*-11 isomer. Furthermore, the *trans*-9, *trans*-11 CLA isomer did not have any impact on body composition. *In vitro*, *trans*-10, *cis*-12 CLA reduced lipoprotein lipase activity, intracellular triacylglycerol and glycerol, and enhanced glycerol release into the medium [Park *et al.*, 1999b], however, *cis*-9, *trans*-11 and *trans*-9, *trans*-11 isomers of CLA failed to have an impact on these components. Baumgard *et al.* [2000] reported that the active isomer of CLA causing milk fat depression or a fall in mammary lipogenesis was the *trans*-10, *cis*-12 CLA. No impact of infusion of *cis*-9, *trans*-11 CLA was observed. Expression [Lee *et al.*, 1998] or activity [Bretillon *et al.*, 1999] of hepatic stearoyl-CoA desaturase was not altered by the *cis*-9, *trans*-11 CLA, but the activity [Bretillon *et al.*, 1999] of the enzyme was reduced by the *trans*-10, *cis*-12 CLA. Therefore, all isomers of CLA are not equal in terms of their influence on lipid metabolism. Further studies are necessary for the evaluation of the biological effects of CLA with respect to alteration in the degree of adiposity and attributes of CLA. Fat deposited under the influence of CLA feeding may be related to changes in expression of key genes for lipid metabolism. Specifically, decreased expression of sterol-CoA desaturase may increase the saturated fatty acid concentration in adipose tissue of pigs, leading to increased firmness of fat associated with bacon.

CLA AND BONE HEALTH

Most studies show an increase in percent of ash when CLA is fed to chicks. This effect is presumed to be due to protection conferred by CLA on bone loss. An increase in cytokines increases bone loss and CLA appears to counter the effect of cytokines. Rodents fed butter had a greater trabecular bone formation (most important for the prevention of osteoporosis) than animals fed vegetable oil.

It has been well documented that lipids have an important role in modulating skeletal biology and bone health. For instance, Wuthier [1993] stated that phospholipids increase cartilage mineralization in growth plate, and a review by Marks & Miller [1993] indicates that signals from biomechanical forces are mediated by prostaglandins (PGs). The PGs play an important role in regulating anabolic factors, such as insulin-like growth factors (IGFs) to support bone formation. Epidemiological evidence from both animal and human studies has shown that dietary lipids have an impact on bone modelling and remodelling. In addition, these researches demonstrate that dietary fat intake is associated with reduced risk of vertebral and femoral fractures in adults and saturated fat intake caused an increase in bone density in children [Gunnes & Lehmann, 1995]. Studies with chicks and rats showed that polyunsaturated fatty acids (PUFAs) and CLA influenced histomorphometric measurements of bone modelling [Li *et al.*, 1999; Watkins *et al.*, 1996; 1997].

Dietary fat can influence bone formation by modulating the production of PGs. The PGs, which are locally synthesized from 20-carbon essential fatty acid precursors, that is, 20:4(n-6) and 20:5(n-3) in osteogenic cells, regulate both bone modelling and bone resorption [Marks & Miller, 1993]. Watkins *et al.* [1996; 1997] as well as Watkins & Seifert [2000] reported that dietary lipids, mainly n-3 fatty acids and CLA, modulated the production of PGE_2, altered the concentration of IGF-1 in bone tissues and led to increased or decreased bone formation rates in growing chicks and rats (mechanisms proposed in this process are presented in Figures 4 and 5).

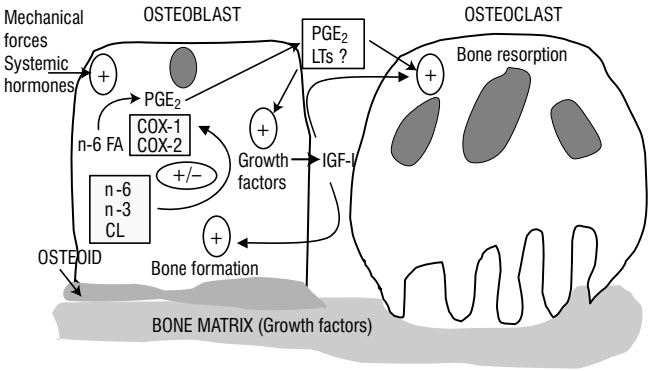

FIGURE 4. Observed effects of dietary fatty acids and related compounds on osteoblastic and osteoclastic activity in bone. Excessive biosynthesis of PGE_2 may depress bone formation and lead to increased bone resorption. Altering the production of eicosanoids (PGE and LTB) appears to optimize formation by influencing IGF-1 production and action [Watkins & Seifert, 2000].

In the studies by Watkins *et al.* [1996; 1997], rats offered a supplement of CLA revealed a decline in the rate of bone formation, suggesting a down-regulating effect on osteoblastic activity. McCarthy *et al.* [1991] showed that PGE_2 produced by osteoblasts may stimulate IGF-1 synthesis or affect its activity to promote anabolic processes in the bone. Research with fats derived from dairy products demonstrated that butter fat – one of the natural sources of CLA blended with corn oil – diminished *ex vivo* bone PGE_2, increased bone IGF-1 level and augmented the rate of bone formation in animals almost by 60% compared to animals offerred diets higher in n-6 fatty acids [Watkins *et al.*, 1997]. The results of this experiment show that decreasing the activity of n-6 fatty acids by n-3 fatty acids and CLA can be of benefit in bone modelling.

So far, all experiments with the use of PUFAs on bone modelling in animals have found that the intake of different PUFA families and CLA can alter directly bone formation *via* modulation of PGs production or indirectly by their influence on IGFs synthesis. The anabolic effects of PGE_2

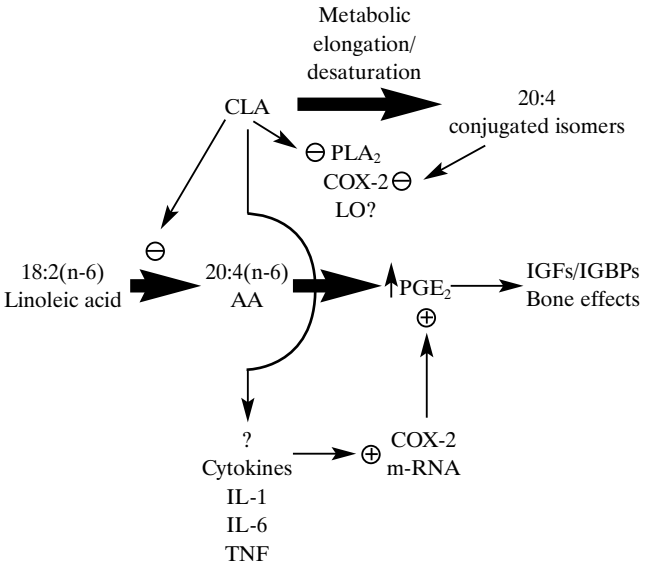

FIGURE 5. Proposed mechanisms for the actions of CLA on PGE_2 production (from AA) and bone metabolism. Black arrows indicate biochemical reaction processes where CLA and linoleic acid participate. Line arrows indicate possible effects of CLA and its metabolites on PGE_2 metabolism and their subsequent action on bone (⊖, inhibition of a process; ⊕, stimulation of a process). Phospholipase A_2=PLA_2. Lipoxygenase enzyme = LO [Watkins & Seifert, 2000].

may occur through stimulation of osteoblast endogenous IGF-1 synthesis [Raisz, 1993] or by increasing bone cell responsiveness to IGF-1. Dietary fatty acids, depending on the type (n-3 or CLA) and amount ingested, may therefore increase or decrease IGF-1 production in bone through their ability to alter local concentrations of PGE_2.

Li & Watkins [1998] as well as Li *et al.* [1999] have hypothesised that CLA depresses arachidonate-derived eicosanoid biosynthesis based on reduced *ex vivo* PGE_2 production in rat bone organ culture and liver homogenate. The decline in PGE_2 production by CLA might be explained as a competitive inhibition of n-6 PUFA elongation that leads to lowered substrate availability for cyclooxygenase.

Other possible mechanisms of the action of CLA include diminution in desaturation/elongation of linoleic acid and inhibition of prostanoid production by its isomeric analogues. This has been demonstrated by Sěbědio *et al.* [1997], who reported that CLA may be further desaturated and elongated to form conjugated C20:4 isomers which might block the access of arachidonic acid to cyclooxygenase. The unusual isomers of C20:4 of CLA might also interfere with the activity of cyclooxygenase.

CLA AND ATHEROSCLEROSIS

Unlike saturated fatty acids, linoleic acid and alfa-linolenic acid, there is scarcity of data about the influence of dietary conjugated linoleic acid on plasma lipoproteins and aortic atherosclerosis. Lee *et al.* [1994] were the first to investigate the effect of dietary CLA on the initiation and progression of atherosclerotic lesions in rabbits *via* its impact on lipid peroxidation. They reported that rabbits offered atherogenic diet with the ingestion of 0.5 g CLA per day for the period of 22 weeks had significantly lower plasma triglyceride, LDL-cholesterol (LDL-C) and LDL-C/HDL-C ratio than control animals. CLA supplementation also brought about a fewer aortic fatty lesions. In a later study conducted in hamsters by Nicolosi & Laitinen [1996] it was shown that feeding CLA collectively significantly decreased levels of plasma total cholesterol, non-high-density lipoprotein cholesterol and triglycerides with no effect on high-density lipoprotein cholesterol compared with controls. Hamsters in this experiment developed 45% fewer aortic fatty streaks than their control counterparts. However, the intact linoleic acid and CLA derived from linolenic acid (18:3) did not have any effect on fatty streak formation, though they did have an impact on blood lipoproteins, suggesting that blood lipoproteins do not play a significant role in the mechanism of action of CLA. The studies by Nicolosi & Laitinen [1996] demonstrated that in comparison to the control group, plasma tocopherol/total cholesterol ratios determined from plasma pools for the low, medium and high conjugated linoleic acid and linoleic acid groups were increased by 48%, 48%, 86%, and 29%, respectively, demonstrating a tocopherol-sparing effect, at least for the conjugated linoleic acid treatment. In a recent study in rabbits by Kritchevsky *et al.* [2000], it was shown that CLA, even at levels as low as 0.1% of the diet, inhibited atherogenesis. At 1% of the diet, CLA caused a significant regression of atheroma. In one of the most recent students by Sher *et al.* [2003], it was demonstrated that when CLA was fed concurrently with cholesterol, plasma and liver cholesterol were reduced up to 40% independent of the induction of acute phase response (APR). However, in hamsters not fed dietary cholesterol, CLA exaggerated the rise in plasma and LDL cholesterol during the APR, even in the presence of varying amounts of vitamin E. Therefore, it could be postulated that the putative antiatherogenic activity of CLA might not be due to its anti-inflammatory or antioxidant effect on lipoprotein metabolism. This is because CLA in this study failed to attenuate APR. The mechanism by which CLA influences the development and regression of atherosclerosis remains to be elucidated.

CLA AS AN IMMUNOMODULATOR

Physiological role of CLA in normal and immune-stimulated animals was studied by Cook and Pariza in the early 1990s. Cook [1991] carried out experiments to determine how nutritional methods could prevent growth suppression that is usually observed with immune stimulation in animals, for example, vaccine. Cook *et al.* [1993] studied the ability of CLA to influence growth in baby chicks following immune stimulation with bacterial lipopolysaccharide (LPS, otherwise known as endotoxin). Generally, chicks lose body weight 24 h after being injected with LPS; this is brought about by the action of cytokines secreted and released by immune cells. These immunocytes, mainly interleukin-1 and tumour necrosis factor induce skeletal muscle breakdown. CLA was found to be protective against the growth suppression associated with immune stimulation. Miller *et al.* [1994] showed that splenocytes from mice fed CLA had increased blastogenesis to phytohemagglutinin. These findings have been confirmed by Chew *et al.* [1997] and Wong *et al.* [1997], who found that lymphocyte proliferation in mice fed 0.3% and 0.9% CLA was enhanced in phytohemagglutinin-induced but not in concanavalin A- or

lipopolysaccharide-stimulated cultures. Production of IL-2 was also increased by CLA, but feeding mice with CLA did not have any impact on lymphocyte cytotoxicity.

THE EFFECTS OF CLA ON DIABETES AND INSULIN RESISTANCE

Houseknecht *et al.* [1998] demonstrated that feeding CLA to rats prone to developing diabetes normalised glucose tolerance and improved hyperinsulinemia as effectively as currently used medications. The CLA used in their research was a mixture composed of 90% isomers of CLA with 42% *cis*-9, *trans*-11 and *trans*-9, *cis*-11 CLA, 43.5% *trans*-10, *cis*-12 CLA, 1% *cis*-9, *cis*-11 CLA, 1% *cis*-10, *cis*-12 CLA, 1.5% *trans*-9, *trans*-11 CLA and *trans*-10, *trans*-12 CLA, 0.5% linoleic acid, 5.5% oleic acid, and 5% unidentified compounds. In addition to normalising impaired glucose, CLA significantly reduced epidydimal fat mass and serum leptin levels. These data suggested that CLA was able to delay diabetes through a mechanism targeting adipose issue in the animal model used in this experiment. However, in another study with the mouse model, the ingestion of CLA mixture brought about an increase in insulin secretion and a decrease in glucose clearance, which led to paroxystic conditions of lipodystrophy syndrome [Tsuboyama-Kasaoka *et al.*, 2000]. This effect has been attributed to the *trans*-10, *cis*-12 isomer in female mice [Clement *et al.*, 2002]. In a recent study in obese humans performed by Risérus *et al.* [2002 a], a 19% loss of insulin sensitivity in the *trans*-10, *cis*-12-treated individuals as compared to the placebo was observed. Paradoxically, Houseknecht *et al.* [1998] and Ryder *et al.* [2001] have demonstrated that this is not relevant to the diabetic *fa/fa* obese Zucker rat in which feeding CLA mixture improved glucose tolerance, as much as the antidiabetic drugs, thiazolinediones.

The *trans*-10, *cis*-12 CLA isomer seems to increase the propensity to insulin resistance even in humans. However, the effect of this isomer in interfering with insulin sensitivity is not observed when it is included in a mixture [Risérus *et al.*, 2002b]. Therefore, caution should be taken both in the consumption of CLA by certain groups of the population, and in the choice of the types of CLA isomers used and in the interaction between them.

CONCLUSION

The effects of individual isomers of CLA so far evaluated need to be further explored, especially the ability of individual isomers to alter gene expression and aggravate insulin resistance as well as type 2 diabetes mellitus. Most of the published results deal with both the 9*c*, 11*t*- and 10*t*, 12*c*-isomers, and explain many of the already described effects obtained with complex mixtures of CLA. Data showing that the 9*c*, 11*t*-isomer is the only biologically active CLA are not supported. The influence of individual isomers in preventing or delaying the onset of diseases should be investigated so that the respective roles of CLA isomers in health as well as their toxicological effects are known. This should help to delineate the balance between the risk and the desired benefit while ingesting either isomer, with the knowledge of the possible side-effects.

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WSPÓŁCZESNE POGLĄDY NA TEMAT ROLI SKONIUGOWANEGO KWASU LINOLOWEGO (CLA) W BUDOWANIU ZDROWIA CZŁOWIEKA: MECHANIZMY DZIAŁANIA

Sa'eed Bawa

Zakład Dietetyki, Katedra Dietetyki i Żywności Funkcjonalnej, Wydział Nauk o Żywieniu Człowieka i Konsumpcji, Szkoła Główna Gospodarstwa Wiejskiego, Warszawa

W niniejszej pracy przeglądowej zacytowano ponad 80 prac na temat koniugowanego kwasu linolowego (CLA), jakie ukazały się w ciągu ostatnich kilku lat oraz w roku 2003 w piśmiennictwie światowym. Poruszono zagadnienia związane z występowaniem CLA w żywności, metabolizmem i znaczeniem tego związku w zapobieganiu rozwojowi tzw. chorób cywilizacyjnych, takich jak otyłość, miażdżyca, nowotwory złośliwe, cukrzyca typu 2, insulinooporność oraz w modulacji czynności układu odpornościowego, z uwzględnieniem mechanizmów działania tego związku. Ponadto, na podstawie analizowanych prac i badań naukowych, omawiano potencjalne zagrożenia „nadużywania" CLA w postaci preparatów syntetycznych.