



## CONJUGATED LINOLEIC ACID: SOURCES, BIOSYNTHESIS AND HEALTH IMPLICATION

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### ABSTRACT

Conjugated linoleic acid (CLA) collectively refers to a group of positional and geometric isomers of linoleic acid with conjugated double bond structure arranged in different combinations of *cis* and *trans* configuration. Importance of CLA in the diets of humans as a vital health promoter has accomplished significance recently. It is present in many foods, especially high in milk and meat of ruminants, has numerous health benefits. Beneficial effects of CLA includes anticarcinogenic, anti-atherogenic, anti-obesity, anti-diabetic, immune system enhancement and few of detrimental affects includes gastrointestinal disorders, insulin resistance and gallstone formation which are isomer specific. In spite of numerous health benefits associated with CLA, their mechanism of action still remains controversial. So, it is important to understand how different isomers of CLA functioning in the body acts to develop CLA enriched functional foods of animal origin. The objective of this review is to provide an overview regarding factors affecting CLA composition, its synthesis in ruminants and its impacts on human health.

**KEY WORDS:** CLA, isomers, milk, meat, functional foods and health impact

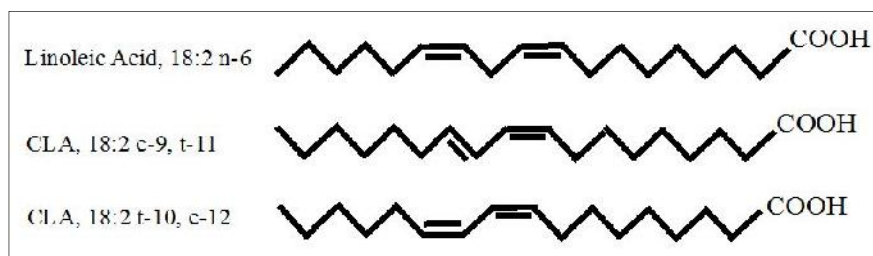
### INTRODUCTION

Animal products contribute significantly to the total nutrients in our food supply. Food products derived from animals has micro components in addition to their nutritive values that have helpful effects on human health and disease prevention (Molkentin, 1999). Conjugated linoleic acids represent one of these micro components of animal origin in human diet. Near about 60% of naturally occurring CLA is found in dairy products, 37% in meats and the rest in processed foods. CLA was granted "Generally Recognized as Safe", status recently in the United States (GRN no. 232; <http://www.cfsan.fda.gov/>) for use as a dietary supplement. The presence of fatty acids with conjugated double bonds was first demonstrated in food products derived from ruminants by Booth *et al.* (1935). However, Michael Pariza in 1978 first discovered its function to inhibit chemically induced cancer in mice. From then several research was conducted to study their positive attributes and adverse health impacts in animal model as well as in human. Despite their several

physiological effects on health, the mechanism of action is yet to be explored precisely.

### Chemistry of Conjugated Linoleic Acid

CLA comprises a family of positional and geometric isomers of linoleic acid with two conjugated unsaturated double bonds, one occurring at position 9 and another at 12 position in the carbon chain, both in the "*cis*" (meaning on the same side) or "*trans*" configuration (Figure 1). Thus, the chemical structure of linoleic acid is *cis*-9, *cis*-12 octadecadienoic acid. There are 28 possible isomers of CLA, each one with a slightly different arrangement of chemical bonds. The major isomer of CLA in milk fat is *cis*-9, *trans*-11 or "ruminic acid" that represents more than 80% of the total CLA (Bauman and Griinari, 2001) and are valuable for the health (Park and Pariza, 2007). *Cis*-12, *trans*-10 is another common isomer of CLA which accounts for 1–10% of total CLA from dietary sources (Choi *et al.*, 2004) and is associated with the anti-obesity effects (Park *et al.*, 2007).



**FIGURE 1:** Chemical structure of CLA isomers

**Dietary Sources of Conjugated Linoleic Acid**

Products of ruminant origin are rich source of CLA which is mostly found in animal fat. There are bacteria in intestine of non ruminants like humans that could also produce CLA from vaccenic acid by  $\Delta^9$  desaturase (Chilliard *et al.*, 2003). Among all foods, kangaroo meat has the highest concentration of CLA. Some mushrooms,

such as *Agaricus bisporus* and *Agaricus subrufescens*, are rare source of CLA. The amount of CLA present in common foods is given in table 1. CLA isomers can also be commercially produced by heating linoleic acid in the presence of alkali or by partial hydrogenation of linoleic acid (Banni, 2002).

**TABLE 1: CLA concentration in various food items (mg)**

Food	CLA per gram of total fat (mg)	Food	CLA per gram of total fat (mg)
Dairy Products		Meats/Fish/Egg	
Homogenized cow's milk	4.8 – 5.5	Lamb	5.6
Condensed milk	7.0	Fresh ground beef	4.3
Butter	4.7	Veal	2.7
Butter fat	6.1	Chicken	0.9
Cottage cheese	4.5	Pork	0.6
Cultured butter milk	5.4	Fresh ground turkey	2.6
Ice cream	3.6	Salmon	0.3
Sour cream	4.6	Egg yolk	0.6
Frozen yogurt	2.8	Vegetables	
Medium cheddar	4.1	Safflower oil	0.7
Plain yogurt	4.8	Sunflower oil	0.4

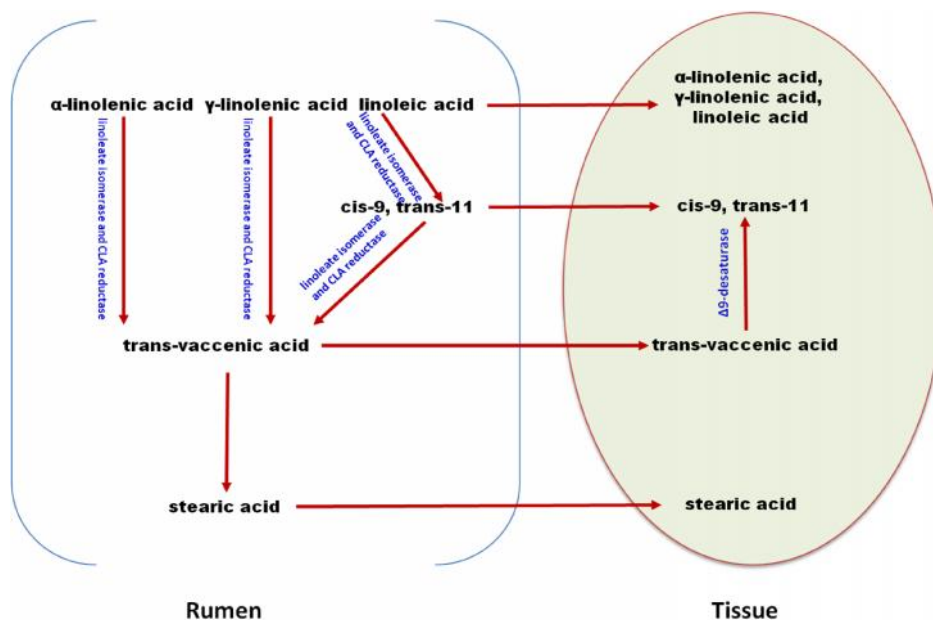
**Factors affecting composition of CLA**

Composition of CLA is influenced by diet, species, breed, variation between individual, age etc. Among these, diet strongly influences the CLA content in milk and meat. For optimal CLA production, cow need to graze on grass rather than be artificially fattened in feed lots (Collomb *et al.*, 2004). Rego *et al.* (2004) also reported higher concentration of milk fat TVA and CLA on pasture grazing as compared to cows fed a diet consisting of corn silage (60%) and concentrate (40%) on dry matter basis. A comparison of feeding a mix of barley and silage to entirely pasture has shown an increase in the CLA content of meat from 3 to 14 mg/gm of fat (Poulson *et al.*, 2004), besides, CLA content also increased when meat was heated to a temperature above 80°C (Dhiman *et al.*, 2005). Milk from high altitude (3700-6200 ft) and organic pasture grazers has more CLA with significantly less saturated fat (Collomb *et al.*, 2004). Further, Tyagi *et al.* (2007) reported a 3 fold increased in total CLA content in milk of cows and buffaloes fed berseem fodder compare to concentrate. Rumen pH influences the composition of CLA in milk, in agreement with Qiu *et al.* (2004) who also observed decreased ruminal pH is inversely proportional to CLA concentration in milk, which might be due to decreased cellulolytic bacterial activity at lower pH reducing biohydrogenation and thereby increasing CLA. Composition of CLA is very low in non-ruminants compared to ruminants. Breed and age also influences CLA content in milk (Dhiman *et al.*, 2002), who reported a higher CLA content in milk of Holstein Friesian compared to Jersey cows, older cows (>7 lactations) have higher CLA compared to younger cows, which might be due to differences in denaturise enzyme activities, fatty acid metabolism and CLA synthesis. Research have reported a three to four fold increases in CLA content in milk through feeding of oil seeds rich in linoleic acid, such as sunflower, soybean, peanut, canola and linseed

(Collomb *et al.*, 2004). The addition of tannins in diets also revealed improves milk yields and increase CLA concentrations in ruminant derived foods (Patra and Saxena, 2011).

**Biosynthesis of CLA**

Conjugated Linoleic Acid found in milk and meat of ruminants originates in two pathways that include formation of CLA during ruminal biohydrogenation of linoleic acid and synthesis of CLA by the animal's tissues from trans-vaccenic acid (*trans*-11 C18:1). Naturally occurring CLA originates mainly from bacterial isomerisation or biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen and the desaturation of trans-fatty acids in the adipose tissue and mammary gland (Jenkins *et al.*, 2008). The enzymes linoleate isomerase and CLA reductase present in bacteria, *Butyrivibrio fibrisolvens* (Kepler *et al.*, 1966) transfers  $\Delta^9$ -linolenic acid,  $\Delta^9$ -linolenic acid and linoleic acid to trans-vaccenic acid in the rumen which is then hydrogenated to stearic acid in the tissue by other ruminal bacteria (figure 2). Isomerization and biohydrogenation of CLA is strongly affected by the rumen pH as decreased rumen pH can result in a shift of the bacterial population, which then influences the pattern of the fermentation and hence the end products obtained as a result. Kishino *et al.* (2002) found *Lactobacillus plantarum* formed high levels of CLA from free linoleic acid upon extended incubation. Several other bacteria such as *Bifidobacteria*, *Lactobacillus acidophilus* and *Megashphaera elsdonii* also produce CLA, mainly the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomer. Furthermore, Xu *et al.* (2008) also reported the production of CLA isomers from linoleic acid by different lactic acid bacteria grown in MRS agar and skim milk. However, major proportion of CLA in tissue and milk lipids synthesized endogenously from *trans* vaccenic acid (TVA) by  $\Delta^9$ -desaturase in mammary gland.



**FIGURE 2:** Biosynthesis of cis-9, trans-11 Conjugated linoleic acid in ruminants

Griinari *et al.* (2000) reported the contribution of endogenous synthesis to the overall CLA content in milk fat was 84%, making it the primary contributing source. Further, Lock and Garnsworthy (2002) also observed that the endogenous synthesis of CLA was more than 80% of total collected duodenal samples and estimated the rumen synthesis of CLA was only 4-7%.

#### **Beneficial properties of conjugated linoleic acids**

Beneficial effects of CLA on health are discussed in a brief as under

#### **Anticarcinogenic properties**

The type of CLA most abundant in meat and dairy products (cis-9, trans-11, CLA) appears to be the champion cancer fighter. Several studies in animal models reported that CLA inhibits chemically- induced tumors of the mammary gland, skin, colon, as well as peritoneal metastasis of human breast, stomach, prostate and colon cancer cells in mouse and rat models (Larsson *et al.*, 2005; Bhattacharya *et al.*, 2006; Hsu *et al.*, 2010; Oh *et al.*, 2014). High intake of high-fat dairy foods and CLA reduced the risk of colorectal cancer (Larsson *et al.*, 2005). Mechanisms of CLA on cancer prevention might be due to modulation of eicosanoid production, interference in cell signalling pathways, inhibition of DNA synthesis, promotion of apoptosis and modulation of angiogenesis (Bhattacharya *et al.*, 2006; Hsu *et al.*, 2010). CLAs have been implicated in the attenuation of all the 3 major stages of development of cancer (initiation, progression and metastasis), particularly with regards to mammary cancer in rodent models (Belury, 2002). Ohtsu *et al.* (2005) demonstrated that CLA decreases cellular proliferation and inhibits NF- B and activator protein-1 activation in PC3 cancerous prostate epithelial cells. Kathirvelan (2007) reported that feeding CLA enriched ghee to female wistar rats lowered around 37% gross tumors incidence in 7, 12 dimethyl benz (a) anthrazene induced mammary gland carcinogenesis. Blewett *et al.* (2009) reported that treatment with vaccenic acid inhibits the growth of human colonic adenocarcinoma cells. More recently, Oh *et al.*

(2014) reported in a separate study with both TVA and c9, t11-CLA (50–200  $\mu$ M) significantly decreased breast carcinoma cells.

#### **Antioxidant activity**

Antioxidant activity of CLA was first recognized in 1990 through in vitro studies (Ha *et al.*, 1990) where they reported CLA as an effective antioxidant, more potent than -tocopherol and almost as effective as butylated hydroxytoluene (BHT). Kathirvelan (2007) observed that feeding CLA enriched ghee in rats resulted in increased antioxidative as well as detoxifying enzymes (Catalase, Superoxide dismutase and Glutathione S-transferase) in liver and mammary gland. Choi *et al.* (2007) also observed that cis-9, trans-11 CLA enhanced hepatic mitochondrial function and protects against oxidative stress through an increase activity of mitochondrial antioxidant enzymes.

#### **CLA and body weight**

Conjugated Linoleic acids have multiple anti-obesity mechanisms such as, reducing food intake, modulating lipid metabolism, increasing fatty acid -oxidation and increased metabolic rate (Park, 2009; Park and Pariza, 2007). CLA isomers such as trans-10, cis-12 reduced body fat in mice from 60% to 80% (Ide, 2005), whereas modest and inconsistent (6% to 25%) in pigs (Qi *et al.*, 2014) and in hamsters ranges from 9% to 58% (Miranda *et al.*, 2009). Rahman *et al.* (2000) observed in 4-week-old rats supplemented with diet containing CLA resulted in reduction of 42% serum leptin concentration and a 5.2% decrease in body weight. Gaullier *et al.* (2005) reported in human's studies supplemented with 3.4g CLA/d in the triglyceride form for 24 months decreased body fat mass.

#### **CLA and lipid metabolism**

Various studies reported that CLA affects lipid metabolism. CLA significantly reduce plasma low density lipoprotein cholesterol, and triglycerides with no effect on high density lipoprotein cholesterol. In lactating cows, CLA isomer *i.e.* trans-10, cis-12 resulted in a 42% reduction of milk fat synthesis and 44% reduction in milk fat (Baumgard *et al.*, 2000). The ability of CLA to alter

lipid metabolism might be due to induce apoptosis, which is programmed cell death (Ip *et al.*, 2000), increase lipolysis and fatty acid oxidation and reduce tissue uptake of fatty acids (Noone *et al.*, 2002). CLA reduces the body fat by reducing lipase activity and increased lipolysis which enhances fat mobilisation and oxidation (Keim, 2003). Jourdan *et al.* (2009) studied lipogenic genes (ACC1, ACC2, FASN and SCD1) in mice fed with trans-10, cis-12 CLA and reported a reduction in expression level of these lipogenic genes. CLA supplementation decreases adipose mass and increases bone mass in mice were reported (Ing and Belury, 2011) which could be probably through modulation of nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) activity. More recently, Ramiah *et al.* (2014) also reported lower transcriptional level of adipogenic genes PPAR $\gamma$  and aP2 in 2.5-5 % CLA treated broilers results to a decreased proportion of adipose cells and monounsaturated fatty acids (MUFA).

#### **Antidiabetic properties**

The incidence of diabetes in the wealthy societies is increasing hugely. World Health Organization (WHO) published estimates that the diabetic population is likely to increase to 300 million or more by the year of 2025. There is evidence that CLA may have the ability to normalize glucose metabolism. CLA lowers insulin resistance and prevent onset of diabetes by improving glucose homeostasis and inhibiting body fat accretion. Antidiabetic property of CLA is through increased oxidative stress (Riserus *et al.*, 2002). However, anti-diabetic effects of CLA are isomer specific. Several researchers reported that c9, t11-CLA isomer has anti-inflammatory, insulin sensitising effect or reduction of blood glucose levels and t10, c12-CLA isomer is associated with weight loss and reduction of fat stores (Wang and Jones, 2004), cis-9, trans-11 CLA also promotes insulin sensitivity (Halade *et al.*, 2010) by reducing adipose inflammation (Reynolds and Roche 2010). Significant production of CLA was observed in fermented milk containing *L. acidophilus* and *L. casei* which have anti-diabetic activity (Yadav *et al.*, 2007).

#### **Cardio-vascular disease and CLA**

Conjugated Linoleic acids plays an important role in reduction of cardiovascular disease. The possible mechanism for the antiatherogenic properties of CLA in animal models studies might be due to reduction of apolipoprotein-B secretion (Yotsumoto *et al.*, 1999). Feeding CLA to rabbits in various amounts resulted in a dose-dependent reduction in the severity of cholesterol-induced atherosclerotic lesions in the aorta (Kritchevskya *et al.*, 2004). Both mixtures and individual isomers of CLA have been shown to reduce atherosclerotic lesions and improvement in plasma lipid profiles in hamsters (Zuckerman *et al.*, 2002), rabbits (Wilson *et al.*, 2006) and mice (Jones *et al.*, 2009). In rodents dietary CLA supplementation significantly lowered serum cholesterol and triacylglycerol concentrations but the results were not consistent (Lock *et al.*, 2005). Tricon *et al.* (2004) studied the effects of cis-9, trans-11 and trans-10, cis-12 isomers on blood lipid and suggested that cis-9; trans-11 isomer is associated with reducing blood lipid levels. Kritchevsky *et al.* (2004) also observed 30% regression of atherosclerotic

lesions in rabbits supplemented with CLA. CLA-fed animals had significant regression of atherosclerotic plaque and decreased macrophage infiltration into lesions despite no change in plasma cholesterol. This was accompanied by increased concentrations of nuclear receptors PPAR $\gamma$  and PPAR $\alpha$  in atherosclerotic lesions that suppress the development of atherosclerosis (Park, 2009). However, Eftekhari *et al.* (2014) reported supplementation of CLA did not significantly affect HDL cholesterol, LDL cholesterol, and total cholesterol in coronary atherosclerosis patients.

#### **Immune modulating properties**

Conjugated Linoleic acids has potent immunomodulatory properties that are exhibited in an isomer specific manner. CLA isomers trans-10, cis-12 is largely responsible for induction of inflammatory response in adipose tissue, by activating integrated stress response leading to activation of NF-k B pathway, induction of inflammatory cytokines, TNF- $\alpha$ , IL-6, and IL-8 (LaRosa *et al.*, 2007), and macrophage infiltration (Liu *et al.*, 2007). Yu *et al.* (2002) reported that CLA exhibits anti-inflammatory effects by negatively regulating the expression of certain pro-inflammatory genes. Roos *et al.* (2005) reported anti-inflammatory role of cis-9, trans-11 CLA related to induction of anti-inflammatory heat shock protein (HSP) 70 kDa and decreased expression of pro-inflammatory macrophage migration inhibitory factor. Bassaganya-Riera and Hontecillas, (2006) reported in pig fed with CLA enriched diet reduced colitis induced weight loss. Ramirez-Santana *et al.* (2011) also observed enhancement of antibody synthesis and lowering the proliferative ability of splenocytes in rats by feeding cis-9, trans-11 CLA during early life.

#### **Deleterious effects associated with supplementation of CLAs**

Despite numerous health benefits CLA is also associated with some adverse health effects.

- The reports of adverse effects in human subjects are limited, with the most common being were of gastrointestinal origin like diarrhea, stomach pain, nausea, flatulence (Wadstein and Gudmundsen, 2000).
- Large dose of supplemental CLA caused increase fatty liver, which is a predisposing factor for various metabolic syndrome and diabetes (Riserus *et al.*, 2002, Cooper *et al.*, 2008). Several researchers reported the cause of diabetes was mainly by trans-10, cis-12 isomer (Khanal, 2004; Wang and Jones, 2004; Stout *et al.*, 2011). Stout *et al.* (2011), reported increases in membrane associated *protein kinase C* (PKC) during trans-10, cis-12 CLA-induced hepatic steatosis. Furthermore, these increased PKC affects insulin signalling leading to insulin resistance, hyperinsulinemia and hyperglycemia (Stout *et al.*, 2011). However, rapeseed oil and olive oil prevented the hepatic steatosis if supplemented along with CLA in mice (Scalerandi *et al.*, 2014).
- Supplementation of CLA resulted in reduction of fat mass by adipocyte apoptosis, caused insulin resistance and marked hepatomegaly characteristic of lipodystrophy in mice. The investigators concluded that leptin deficiency may explain the insulin resistance, as leptin functions to normalize blood

insulin concentrations and reduce fat deposition in the liver (Tsuboyama-Kasaoka *et al.*, 2000).

- There are also numerous studies, both in animals and humans, showing that despite lowering body fat, CLA can drive inflammation and lower beneficial HDL cholesterol (Khanal, 2004). Riserus *et al.* (2004) also reported an increased in lipid peroxidation and multiple inflammatory markers in overweight men supplemented with CLA.
- C-reactive protein (CRP) is a protein produced by the liver, which rises during widespread inflammation. It is associated with formation of blood clots and atherosclerosis. Supplementation of CLA in dietary could cause an increase in the levels of C-reactive protein and indicates an increased risk for heart attack and stroke (Khanal, 2004; Vyas *et al.*, 2012).
- An increase in distal colon carcinogenesis, but no effects in proximal colon or caecum in ApcMin mice fed the trans-10, cis-12 isomer (Rajakangas *et al.*, 2003).

## CONCLUSION

There are 28 types of isomers of CLA out of which cis-9 and trans-11 is predominant associated with anticarcinogenic, antiatherogenic, antidiabetogenic and immune modulating properties. While other isomers of CLA, isomer trans-10, cis-12 is negatively associated with milk fat synthesis and body fat accretion. However, all these findings were based on research absolutely in animal models and the mechanism(s) by which they exerted their effects remain largely unknown. Thus, while consideration of functional foods containing CLA represents an exciting research area. Further research giving greater insight not only into the effectiveness of CLA, but also to the specific physiological and biochemical pathways that are responsible for understanding how it works in the human body is need of the hour.

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