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*Review*

# Isomer specificity of conjugated linoleic acid (CLA): 9*E*,11*E*-CLA

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## Abstract

Conjugated linoleic acids (CLA) were identified in 1980's, since then it has been intensively studied due to its various beneficial health effects such as anti-inflammatory, anti-atherogenic, anti-carcinogenic and anti-diabetic/obesity effects. Isomer specificity of a number of CLA isomers, especially predominant isomer 9*Z*,11*E*- and 10*E*,12*Z*-CLA, is now recognized. However, the less prevalent CLA isomers have not been well characterized. Recently, studies have reported the distinctively different effects of 9*E*,11*E*-CLA in colon cancer cells, endothelial cells, and macrophage cells compared to the rest of CLA isomers. In this review, various effects of CLAs, especially anti-inflammatory and anti-atherogenic effects, will be discussed with focusing on the isomer-specific effects and potential mechanism of action of CLA. At last, recent studies about 9*E*,11*E*-CLA in *in vitro* and animal models will be discussed.

**Key Words: Conjugated linoleic acid (CLA), 9*E*,11*E*-CLA, anti-inflammatory, isomer-specificity**

## Overview of conjugated linoleic acids

CLAs are fatty acids that are mainly found in foods derived from ruminant animals (Pariza & Ha, 1990). They are geometrical and positional isomers of its parent molecule, linoleic acid (*cis*-9,*cis*-12-18:2, n-6 (LA)). The *cis*-9, *trans*-11 (9*Z*,11*E*-octadecenoic acid, C18:2) isomer, also known as rumenic acid (RA), is generated via biohydrogenation of dietary linoleic acids by ruminant microflora and is the most abundant natural CLA isomer (over 75-80% of total CLA). It is not clear whether humans are able to produce RA from other fatty acids (Yurawecz, 1999); however, it is likely that the majority of RA found in the human body originates from the diet. Estimated CLA intake for humans is between 0.5 and 1 g/d (Ip *et al.*, 1994); however, more recent estimates suggest that CLA and RA intakes are much lower (Yurawecz, 1999). For example, the average intake is 430 mg/day for men and 350 mg/day for women in Germany, 212 mg/day for men and 151 mg/day for women in the USA, and 500-1,000 mg/day in Australia (Yurawecz, 1999).

Studied for its potential beneficial effects of CLA on animal health have focused on anti-inflammatory, anti-atherogenic, anti-carcinogenic, and anti-diabetic/anti-obesity effects; however, the beneficial effects of CLA in humans is inconclusive (Kelly, 2001; Pariza *et al.*, 2001; Rainer & Heiss, 2004; Wahle *et al.*, 2004; Whigham *et al.*, 2000). It is important to note that a majority of the CLA studies was performed with a chemically synthesized CLA mixture, although in recent years, some were done with relatively pure 9*Z*,11*E*-CLA or 10*E*,12*Z*-CLA isomers. CLA supplements which predominantly contain two major CLA isomers are on the market; however, the safety of CLA is a concern in public health. To obtain beneficial amounts of CLA, one must ingest 10-200 times what can be achieved in diet (Gaullier *et al.*, 2002). Although animal studies on CLA showed that it is reasonably safe and well-tolerated in rats, dogs, and pigs (O'Hagan & Menzel, 2003; Pariza, 2004; Scimeca, 1998), whether the same is true for humans is not known. Gaullier *et al.* (2002) stated that CLA enriched with 9*Z*,11*E*- and 10*E*,12*Z*-CLA may be better for human consumption than one enriched with 8*E*,10*Z*-, 9*Z*,11*E*-, 10*E*,12*Z*-, and 11*Z*,13*E*-CLA. In spite of these conclusions, concerns on CLA safety remain due to its adverse effects which induce fatty liver, insulin resistance, and lipodystrophy in mice and increase C-reactive protein levels, lipid peroxidation, and reduction of milk fat in humans (Pariza, 2004); therefore, it is essential for long-term human studies to make solid conclusions on recommending a proper amount of CLA supplementation to the public. It is also important to consider differences in CLA isomers in terms of both benefits and potential toxicity.

## Anti-inflammatory effects

Levels of fatty acids are known to have a great impact on immune function. Dietary fat intake directly influences the fatty acid profile of blood lipids and cells. The immune-modulating effects of saturated and polyunsaturated fatty acids have been

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reported (Calder & Grimble, 2002). However, the available knowledge about the effects of CLAs on immune function in animals or humans is limited. The inflammatory-modulating effects of CLA are mainly observed in *in vitro* and animal models, while CLA's anti-inflammatory effects are not conclusive in human studies. Chicks and rats fed 0.5% CLA for 4 wks gained weight even after LPS injection, whereas chicks and rats without CLA lost weight. This led to the idea that CLA inhibited the catabolic effects of endotoxin (Cook *et al.*, 1993). CLA fed mice had higher levels of IL-2 compared to control diet fed mice (Hayek *et al.*, 1999; Wong *et al.*, 1997), while TNF-α and IL-6 levels were decreased (Rahman *et al.*, 2007). However, Kelley *et al.* (2002) reported that both 9*Z*,11*E*- and 10*E*,12*Z*-CLA significantly and similarly increased TNF-α and IL-6 levels without altering prostaglandin levels in CLA fed mice.

In *in vitro* models, CLA was positively related to anti-inflammatory effects. For example, Yu *et al.* (2002) showed that CLA reduced TNF-α and IL-6 levels induced by IFN-γ, at least partially by a PPARγ-dependent pathway. CLA induced IFN-γ and IL-2 levels in Juckat T cells (Luongo *et al.*, 2003). Moreover, IL-10 receptor and IL-10 levels were induced by CLA in mouse dendritic cells (Loscher *et al.*, 2005). In human epithelial cells, 9*Z*,11*E*-CLA attenuated cell growth and reduced IL-8 levels while 10*E*,12*Z*-CLA had no effect (Jaudszus *et al.*, 2005).

The results from human studies focusing on anti-inflammatory effects of CLA are inconclusive. Song *et al.* (2005) demonstrated that CLA supplementation could be beneficial in humans (3 g/d CLA for 12 wks) by enhancing IgA, IgM, and IL-10 but inhibiting IgE, TNF-α, and IL-1β. In addition, CLA boosted the hepatitis B vaccination in healthy human subjects although it did not alter other aspects of immune function including NK cell activity, lymphocyte proliferation, and production of TNF-α, IL-1β, IL-6, IL-2, IL-4, IFN-γ, and $PGE_2$ (Albers *et al.*, 2003). In contrast, other human studies showed no significant relationship between CLA and anti-inflammatory effect (Albers *et al.*, 2003; Kelley *et al.*, 2000; Nugent *et al.*, 2005; Ramakers *et al.*, 2005; Ritzenthaler *et al.*, 2005; Tricon *et al.*, 2004a). There were no changes on the levels of $PGE_2$, leukotriene $B_4$, IL-1β, and TNF-α that were induced by LPS in young healthy women (Kelley *et al.*, 2000).

The regulation of immune functions by CLA does not seem to be affected by a single mechanism. CLA affects the production of eicosanoids either directly or indirectly, enhances PPARγ activation, attenuates the NF-κB pathway, or directly decreases pro-inflammatory cytokines to have beneficial effects on inflammation which ultimately influence metabolic syndromes including obesity, insulin resistance, and atherosclerosis (Zulet *et al.*, 2005). CLA decreased the production of eicosanoids such as $PGE_2$, PGF2α, and $LTB_4$ (Li & Watkins, 1998; Sugano *et al.*, 1998), although the reduction of the eicosanoid production was not consistent (Hayek *et al.*, 1999; Kelley *et al.*, 2000). The mechanism by which CLA reduces AA-derived eicosanoids can be explained as follows: (1) CLA displaces AA in phospholipids; (2) CLA competes with LA for desaturation and elongation; (3) CLA or CLA metabolites may act as substrates or antagonists for enzymes involved in the prostaglandin production process; and (4) CLA-derived eicosanoids themselves may have anti-inflammatory properties. The PPARγ subtype is highly expressed in adipose tissue and macrophages (Olefsky, 2001). Most importantly, PPARγ plays a critical role in the regulation of inflammation (Cuzzocrea *et al.*, 2004). Various CLA isomers activate PPARγ in RAW 264.7 cells and decrease pro-inflammatory cytokines induced by IFN-γ (Yu *et al.*, 2002). NF-κB is a transcription factor that is involved in cytokine gene expression, cellular adhesion, cell cycle activation, apoptosis, and carcinogenesis. CLA negatively regulates inflammatory mediators via the NF-κB pathway by inhibiting IκB phosphorylation (Cheng *et al.*, 2004). Finally, CLA could exert direct anti-inflammatory properties by regulating the gene expression of inflammation mediators (Loscher *et al.*, 2005; Luongo *et al.*, 2003; Song *et al.*, 2005), perhaps due to effects on NF-κB or PPARγ.

In conclusion, the anti-inflammatory action of CLA brings potential therapies for diseases such as atherosclerosis, diabetes, cancers, rheumatism, inflammatory bowel disease and obesity. Meanwhile, the lack of understanding regarding CLAs mechanism (s) of action, safety issue surrounding CLA as a dietary supplement, and isomer-specific effects on regulating inflammatory mediators require further studies.

## Anti-atherogenic effects

Atherosclerosis is a complex disease that is influenced in part by inflammation (reviewed in (Ross, 1999)). Interactions between lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall are important contributors for the development of atherosclerosis. CLA-fed rabbits had significantly lower low density lipoprotein (LDL) cholesterol and TG levels as well as a lower ratio of LDL/HDL (high density lipoprotein) and LDL/total cholesterol compared to control diet fed rabbits (Kritchevsky *et al.*, 2000; Lee *et al.*, 1994). In hamsters, CLA reduced total cholesterol, LDL, VLDL (very low density lipoprotein), and TG although there were no changes on HDL levels (Nicolosi *et al.*, 1997). Unlike the results in rabbits and hamsters, the results from mice were somewhat conflicting (Arbones-Mainar *et al.*, 2006; Munday *et al.*, 1999; Nestel *et al.*, 2006; Toomey *et al.*, 2006). A mixture of CLA (9*Z*, 11*E*-CLA:10*E*, 12*Z*-CLA (80:20 blend)) was tested in ApoE-/- mice and showed a suppression of atherosclerotic lesions by decreasing pro- inflammatory genes (i.e., metalloproteinases (MMP)-9 and PECAM-1) and an increase in apoptotic genes (Toomey *et al.*, 2006). Isomer-specificity of regulation of the lipid profile by CLA isomers was shown in a few studies. Mitchell *et al.* (2005) showed that 10*E*,12*Z*-CLA increased HDL levels in 1% CLA fed hamsters. However, Wilson *et al.* (2006) reported that 9*Z*,11*E*-CLA decreased cholesterol whereas

10*E*,12*Z*-CLA increased cholesterol levels in 0.5% CLA isomer fed hamsters. This discrepancy may be due to the relative amount and composition of the CLA mix used in the studies.

Different types of cell lines related to atherosclerosis were used to study the anti-atherogenic effects of CLA in *in vitro* systems. Decreased levels of 6-keto-prostaglandin F(1α) (6k-PGF(1α)), a stable breakdown product of $PGI_2$, were observed in bovine aortic endothelial cells treated with CLA (Coen *et al.*, 2004). CLA increased CD36 (scavenger receptor) in THP-1, human macrophages as well as reduced total lipids, $PGE_2$, $PGI_2$, and cytokine-induced NF-κB binding activity in human smooth muscle cells (Ringseis *et al.*, 2006; Weldon *et al.*, 2004). However, CLA failed to inhibit adhesion molecules which are important for the initiation step of atherosclerotic lesion formation, such as ICAM-1, VCAM-1, and E-selectin induced by TNF-α in human arotic EC (Schleser *et al.*, 2006). Although there were no changes in ICAM-1 and VCAM-1 levels by CLA, CLA inhibited the adhesion activity of THP-1 cells to human umbilical vein EC (HUVEC) (Sneddon *et al.*, 2006).

CLA's fat-lowering effects have been reported in human subjects, although they have not been consistent. Benito *et al.* (2001) showed no alteration of cholesterol, TG, HDL, and LDL cholesterol levels in human on a 3.9 g/d CLA supplementation for 63 days. Desroches *et al.* (2005) reported that healthy subjects on CLA had lower total cholesterol, total cholesterol/HDL ratio compared to the subjects on control diet. On the other hand, Mougios *et al.* (2001) and Gaullier *et al.* (2004) reported a detrimental HDL-lowering effect of CLA mix in humans. Tricon *et al.* (2004b) showed that 10*E*,12*Z*-CLA increased a ratio of cholesterol/HDL, LDL:HDL, and TG levels compared to 9Z,11E-CLA in healthy humans. In spite of isomer-specific effects of CLA on lipid profile, the same group reported a similar effect of those two CLA isomers on immune cell function as inhibiting mitogen-induced T cell activation (Tricon *et al.*, 2004a). The CLA isomer-specificity varies depending on the results observed; therefore, studies on pure CLA isomers are needed to elucidate these questions.

Some proposed mechanisms for the anti-atherogenic and lipid-lowering effects of CLA include roles for PPARs, sterol regulatory element-binding proteins (SREBPs) and stearoyl-CoA desaturase (SCD) (reviewed in (Bhattacharya *et al.*, 2006)). Further studies using each CLA isomer and long-term clinical studies are needed to establish their potential anti-atherogenic effects and mechanism (s) of action.

## 9*E*,11*E*-CLA: its effects and mechanism

The isomer-specific effects of 9*Z*,11*E*-CLA and 10*E*,12*Z*-CLA are now well-recognized (reviewed in (Evans *et al.*, 2002)). However, the less prevalent CLA isomers have not been intensively studied. Recently, more studies have reported the effects of 9*E*,11*E*-CLA in colon cancer cells, endothelial cells, and macrophage cells (Beppu *et al.*, 2006; Lai *et al.*, 2005; Yasui *et al.*, 2007). Lai *et al.* (2005) showed that among the CLA isomers, 9*E*,11*E*-CLA attenuated proliferation of bovine endothelial cells the most by enhancing caspase-3 activity. In the following year, Beppu *et al.* (2006) reported that 9*E*,11*E*-CLA showed the strongest apoptosis among 9*Z*,11*Z*-, 9*Z*,11*E*-, and 10*E*,12*Z*-CLA isomer on colon cancer cells, Caco-2 cells. The same researchers further reported the 9*E*,11*E*-CLA's chemopreventive effect in rats fed 0.1% or 1% 9*E*,11*E*-CLA for 4 wks (Yasui *et al.*, 2007). Ecker *et al.* (2007) showed that 9*E*,11*E*-CLA, but not 9*Z*,11*E*- and 10*E*,12*Z*-CLA, induces SREBP target genes such as ABCG-1 via a SREBP-1c-dependent mechanism in primary human monocyte-derived macrophages. Comparison of five different CLA isomers showed that 9*E*,11*E*-CLA indeed regulates gene expression distinctively different compared to the rest of CLA isomers in a mouse macrophage cell line, RAW 264.7 (Lee *et al.*, 2008). Thus, isomer specificity of 9*E*,11*E*-CLA has been recognized in various cell types.

Although the portion of 9*E*,11*E*-CLA in American beef fat and commercial CLA mixtures ranged from 1.5-3.7% of the total CLA isomers (Kramer *et al.*, 1998; Yurawecz, 1999), 9*E*,11*E*- and 9*Z*,11*E*-CLA were the main CLA isomers in serum isolated from women on 2.1 g of CLA supplementation for 45 days which represented 41% of the CLA total (Petridou *et al.*, 2003). These isomers were also the two major CLA isomers in platelet lipids, 17% and 33% of the total respectively (Al-Madaney *et al.*, 2003). Based on the basal plasma CLA levels in healthy men as 7 μM, the 9*E*,11*E*-CLA concentration is estimated to be in the 1-3 μM range (Huang, 1994). Considering 10-fold higher concentration in local areas, 10-30 μM might be a physiologically relevant level of 9*E*,11*E*-CLA. Large-scale and economically efficient separation of 9*E*,11*E*-CLA is difficult due to its minimal existence in the CLA mixture. Kishino *et al.* (2002, 2003) reported the bacterial production of 9*Z*,11*E*- and 9*E*,11*E*-CLA from ricinoleic acid (12-hydroxy-cis-9-octadecaenoic acid) and linoleic acid. This biological system for 9*E*,11*E*-CLA production may promise the large-scale and selective preparation of 9*E*,11*E*-CLA.

## Conclusion

In spite of intensive studies of CLAs performed over the last two decades, the mechanism of action of each CLA isomer is not well-appreciated yet. In this review, various health-related effects of CLAs were discussed with a focus on superior and/or distinctive characteristics of 9*E*,11*E*-CLA. The limited numbers of recent studies of 9*E*,11*E*-CLA in *in vitro* and *in vivo* studies suggest that this specific CLA isomer appears to have the anti-carcinogenic, anti-inflammatory, and potential anti-atherogenic effects. Although it requires more *in vivo* and clinical studies before making a solid conclusion, understanding the effects of the individual CLA isomer helps us to make a combination of the CLA isomers in dietary supplements to maximize its healthful effects and minimize its harmful effects.

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