Review Article

Potential Health Benefits of Conjugated Linoleic Acid (CLA): An Important Functional Dairy Ingredient

Abstract

Conjugated linoleic acid (CLA) refers to a class of positional and geometrical isomers of linoleic acid (cis-9, cis-12 octadecadienoic acid) having conjugate double bond system. CLA are synthesized in rumen of the ruminants by biohydrogenation of dietary fatty acids; and thus, can be obtained from dairy products as well as from the meat of sheep, lamb and other ruminants. Among the several isomers, c9, t11-CLA isomer is the most biologically active form and accounts approximately 80% of total isomers. A number of clinical and epidemiological studies have demonstrated the role of CLA as anti-atherogenic, anti-inflammatory, anti-oxidative, anti-carcinogenic, etc. Several researchers have suggested the positive association of CLA in weight management, hypercholesterolemia, immunomodulatory functions, and improved bone metabolism.

Keywords: Conjugated linoleic acid; biohydrogenation; anti-carcinogenic activity; anti-atherogenic; anti-obesity

Introduction

Conjugated linoleic acid (CLA) occurs naturally as *trans*-fatty acid which is naturally synthesized from ω-6 essential fatty acid in the rumen and intestine of pastured ruminants like cattle, sheep and goat. Conjugated linoleic acids have conjugated dual bond and consist a class of geometric and positional isomers of essential linoleic fatty acid (cis-9, cis-12). Various CLA isomers may be present in various double bond positions (7–9, 8–10, 9–11, 10–12, 11–13) or geometric orientation (trans-cis, cis-trans, trans-trans & cis-cis) in which cis-9, trans-11 is the most biologically active form. The c9, t11 isomer has been reported to have anti-carcinogenic effects as well as regulating body mass. Other isomers of CLA have also been reported to confer several health benefits such as preventing artery thickening, protecting the heart disease, preventing from cancer,

and modulating immune system. Technically, conjugated linoleic acid is not an essential fatty acid. Unlike other fatty acids, CLA cannot be synthesized by the human body, and thus, needs to be taken from diet such as meat and dairy products.

Sources

The most common sources of natural CLA in the human diet are milk & milk products and meat of ruminant animals. The content of CLA in various products are given in below table (Table 1).

Structure of CLA and other related fatty acids

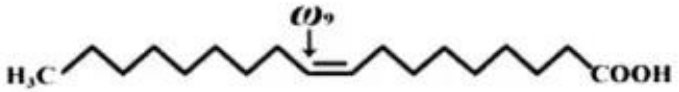
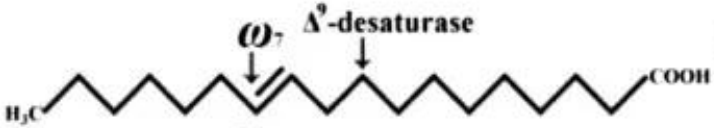
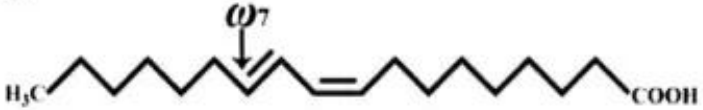
Common/ Trivial name	*ω/n* position (numbering from –CH_3 end)	Structure	Systemic name (numbering from -COOH end)
Oleic acid (OA)	*ω*-9		(*cis*-9)-octadecenoic acid
Linoleic acid (LA)	*ω*-6		(*cis*-9,*cis*-12)-octadecadienoic acid
Vaccenic acid (VA)	*ω*-7		(*trans*-11)-octadecenoic acid
9-CLA (Rumenic acid)	*ω*-7		(*cis*-9,*trans*-11)-octadecadienoic acid

Fig. 1. Structure of CLA and other related fatty acids

Global Market of CLA

CLA is a popular supplement for weight management worldwide. CLA is used for medical benefits like cancer fighting properties, muscle strengthening, improved metabolic functions. Conjugated Linoleic Acid (CLA) market is expected to grow 5.8% CAGR in terms of revenues. The global market size is calculated to reach US$ 50 million up to 2024, from US$ 36 million in 2019. China is the biggest producer of CLA supplements capturing 47.93% market share followed by North America (25.63%).

Biosynthesis of CLA

In ruminants, CLA is synthesized by two methods: 1) Ruminal Biohydrogenation (from linoleic acid), and 2) Endogenous synthesis (in tissues from *trans* Vaccenic acid). First the linoleic acid (C18:2, C9, C11) is converted into conjugated linoleic acid (C9, t11) in presence of linoleate isomerase. Thus, CLA formed goes to mamarry glands and gets incorporated into the milk fat. Some of the portion of CLA is hydrogenated into trans-vaccenic acid (TVA) (t11) (Fig. 2). The trans-vaccanic acid is further hydrogenated into endogenous stearic acid. On the other hand, α-Linolenic acid (ALA) (C18:3) (c9, c11, c15) is converted into Octadecatrienoic acid (C18:3) (c9, t11, c15) in the presence of linoleate isomerase. Hereafter, further conversion is shown in Fig. 2.

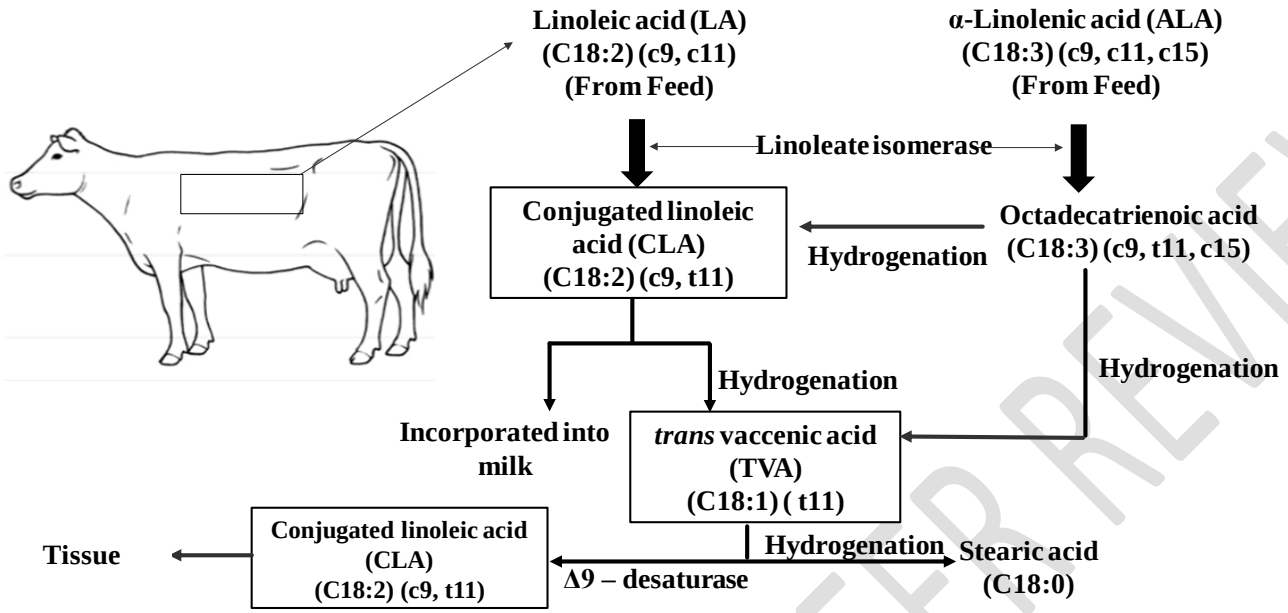


Fig. 2. Biosynthesis of conjugated linoleic acid

Role of rumen bacteria in CLA synthesis

For CLA synthesis, ruminants depend on microbial fermentation of feed and additional forage in rumen. Ruminant diet includes some polyunsaturated fatty acids (PUFAs) which are toxic to many of rumen microorganisms (Harfoot and Hazlewood, 1997). Nevertheless, in order to protect them from toxic effects, rumen microorganisms have pathways for hydrolysis and biohydrogenation of dietary lipid. Rumen bacteria play an important role in biohydrogenation. In biohydrogenation of lipids in rumen, >250 bacterial strains are involved. The most common are:

- *Butyrovibrio*
- *Micrococcus*

- *Lactobacillus*
- *Ruminococcus*
- *Enterococcus*
- *Propionobacterium*

Health benefits of CLA

Several recent clinical and epidemiological studies have reported the important role of CLA as anticarcinogenic, antiatherogenic, immunomodulating, anti-diabetic and lean body mass enhancing, etc., which are discussed below:

Anticarcinogenic activity

Though no concrete evidences are there for the anticarcinogen activity of CLA, but possible mechanisms have been attributed to antioxidant mechanisms, pro-oxidant cytotoxicity, suppression of nucleotide biosynthesis, decrease in proliferative activity and inhibition of carcinogen activation (Mohammadzadeh et al., 2013; Cheng et al., 2019; Kim et al., 2015; Pierre et al., 2013; Soel et al., 2007; Evans et al., 2010). CLA is reported to show prooxidant activity in which they inhibit the oxidation causing substances (Devery et al., 2001). A few studies have demonstrated that CLA decreases the proliferative activity or supress the multiplication of free radical formation (Coakley et al., 2006; Kadidrareddy et al., 2016). In another study, it is suggested that CLA supresses the nucleotide synthesis and thus, inhibit the DNA replication in cells which prevent the formation of tumor in body. Recent clinical and epidemiological studies on the anti-carcinogenic properties of CLA are shown in Table 2.

Antioxidant and anti-inflammatory properties

CLA is more potent antioxidant than other antioxidants. It is more effective than butylated hydroxytoluene (BHT) & alpha-tocopherol. Furthermore, it is reported to be more potent than vitamin E and butylated hydroxy anisole (BHA) in supressing the formation of thio-barbituric

acid reactive substances (TBARS). CLA is one of the biomarkers which is also used to assess oxidation status in biological systems. Environmental mutagens or carcinogens act as inhibitor and they block the carcinogenesis and show their antioxidant properties (Lokesh & Cunningham, 1983). Superoxide dismutase and catalase are the antioxidative enzymes which are involved into the removal of toxic free radicals which leads to amelioration of oxidative damage in cells. Glutathione-S transferase is involved in biotransformation of carcinogens, detoxification of xenobiotics, peroxides and free radicals by conjugating toxic component with GSH (Glutathione reduced) and due to this they ultimately protecting organ and cells against induce toxicity (Basiricò et al., 2015). For this reason, increasing this enzyme by synthetic or natural way, which results in the inhibition of hepatic cancer-causing substances; and CLA behaves like chemo-preventive agent, inhibits the oxidative stress & reduce the levels of detoxifying enzymes (Ip et al., 1999). It is reported that CLA at the level of 0.25% or more in diet inhibits the mammary carcinogenesis and decreases the formation of TBARS into mammary tissue (Kritchevsky, 2016). Kathirvelan (2007) observed that CLA fortified *ghee* (clarified butter) increased the antioxidative activity and reduced the toxic causing enzymes in mammary gland and liver than that of soybean oil. Several clinical and epidemiological studies on the anti-inflammatory and immunomodulatory properties of CLA are presented in Table 3.

Cholesterol-lowering and anti-obesity effects

Several recent animal studies have demonstrated a positive role of CLA in prevention of cardiovascular diseases, hypercholesterolemia and obesity (Wannamethee et al., 2018; Gray et al., 2015; Limongi et al., 2018), which are presented in Table 4. Lee et al. (1994) reported that CLA was effective against different stage of cancer like progression and initiation and found anti-atherogenic effect of CLA (at 0.5 g/d) in rabbits in a study of 22 days. On the other hand, Nicolosi et al., (1997) reported that the hamsters fed together with CLA had significantly decrease the amount of total plasma cholesterol when compared to control. Kathirvelan (2007) observed that the high and low CLA intakes reduced the total cholesterol, and triglycerides, and increased HDL cholesterol when compared with control (soya oil fed rats). Kathirvelan (2007) demonstrated

that the antiatherogenic effects of CLA were shown by decrease in triglycerides, total cholesterol, atherogenic index & LDL cholesterol and a rise in HDL-cholesterol into blood plasma.

CLA and Bone metabolism

Watkins et al (1999) have reported a high level of bone formation in butterfly-fed chickens, which proposed to be likely due to higher CLA consumption. Dietary CLA contributed to variations in CLA abundance in bone marrow, specific tissue and organs, and periosteum having the highest amount of CLA and the low amount in the heart. CLA has been reported to increase cis-9, trans-11 in tissue lipid and also involved in bone biomarkers and development.

Methods to increase CLA concentration in dairy products

There are two methods of CLA enrichment: 1) Dietary modification of animal feed, and, 2) Direct enrichment of milk and dairy products. In dietary modification of animal feed, feed can be supplemented with rapeseed, cottonseed, soybean, corn, peanut, sunflower, canola, safflower and linseed or their oils. Oils can be added into the diet in the form of protected oils, free oils, processed oilseeds or whole oilseeds (extruded, crushed, roasted or ground). These seeds/oils are high in linoleic or linolenic acid, which are precursors of CLA. Oil to be added in diet can be protected in the form of fatty acyl amides, calcium salts, a lipid encapsulation or formaldehyde-protein protection matrix.

Food applications

CLA-rich oils can be used as functional ingredients in a variety of foods such as milk, yoghurt/*dahi*, cheese and *paneer*, milk-based fermented beverages, ice-creams, grains and pasta products, processed fruits and fruit juices, etc. Campbell et al., (2003) fortified CLA oil (Clairinol G-80) in skim milk at the concentration of 1 & 2%. After HTST pasteurization, the concentration of cis-9/trans 11 isomers remains stable by 2 weeks of refrigerated storage. Sensory analysis revealed that in fortified milks, low intensities of a “grassy/vegetable oil” odor was perceived.

Rodriguez-Alcala and Fontecha (2007) supplemented milk powder, milk, yoghurt, fermented milk, fresh cheese and milk-juice blend with Tonalin-80 (CLA-oil, 80% CLA). Major of CLA isomers were not affected by thermal treatment and processing but the total CLA content decreased in fresh samples after 10 weeks of low temperature storage.

Limitations

As far as CLA is concerned, most of the studies have been conducted on animals (rodents, pigs, dogs, etc.) to establish the health effects of CLA. A very few studies have been conducted on human beings. No clear/concrete mechanisms are established yet for its antioxidative, anticarcinogenic and weight-control activities. No RDA is given by any regulatory agency yet. Toxicological effects of CLA are lacking due to insufficient data and research. Further clinical studies are required to establish safe limit or dietary intake of CLA along with the health benefits.

Conclusion

Conjugated linoleic acid have unique property; unlike the other naturally coming anti-carcinogenic substances mainly found in the plants, food from animal sources also contain CLA. The richest isomer is rumenic acid (C18:2 *cis* 9, RA, *trans* 11). CLA isomer: *trans* 10, *cis* 12 is connected with weight management in humans. According to animal studies, 3 g/day of CLA dosage into diet have positive biological effects. However, no RDA is reported yet. Milk fat is one the best natural origin of CLA and would be improved via the modification of animal diets. Diverse physiological functions of CLA in preventing the risks of cancer, obesity, hypercholesterolemia, atherosclerosis, etc. studied in animal model are little convincing and require further intense research. However, a few attempts have been made to confirm the positive impacts of CLA on human; nevertheless, it is not clear whether CLA promotes all of these advantages to humans as well. Natural fortification of food products by modification of animal food that accounting to the overall goal of achieving the potential health benefits of CLA in the future. Further research is required to establish the biological benefits of CLA's activity, to come about efficient strategies of intake and recommended amount for human safety.

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Table 1. Conjugated linoleic acid content (mg CLA/g fat) in various food products

Dairy products	Mg of CLA/g of fat	Meat products	Mg of CLA/g of fat
Milk	5.5	Veal	2.7
Condensed milk	7.0	Ground beef	4.3
Butter fat	6.1	Lamb	5.8
Cultured milk	5.4	Chicken	0.9
Sour cream	4.6	Pork	0.6
Butter	4.7	Ground turkey	2.6
Ice cream	3.6	Egg yolk	0.6
Yogurt (low fat)	4.4	Salmon	0.3
Plain yogurt	4.8		
Custard yogurt	4.8		
Medium cheddar	4.1		

Source: Chin et al., (1992)

Table 2. Major findings of clinical and animal studies based on anti-carcinogenic activity of CLA

Subject	Duration of feeding	CLA concentration in diet	Results	Reference
Mice	NR	1 g CLA/100 g diet	Dietary CLA supplementation reduced disease activity, decreased colitis, and prevented adenocarcinoma formation in cancer-induced mice. Additionally, a significant decrease was also observed in the percentages of macrophages in lymph nodes and the gene expression for colonic tumor necrosis factor-α.	Evans et al., (2010)
Rectal cancer patients (n=34)	6 weeks	3gCLA/d	On comparison with placebo-group, CLA-group showed significant reductions in the pro-inflammatory marjers [TNF-α (P = 0.04), hsCRP (P = 0.03)], and biomarkers of angiogenesis (MMP-9). However, no significant change was observed in the level of IL-6 than that of control.	Mohammadzadeh et al., (2013)
Rats	NA	NA	Polymeric nanoparticles containing linoleic acid conjugate (NPs) were developed. Results revealed that CLA-NPs were effective in growth inhibition of human colon cancer cells. Another important observation was that CLA-NPs could avoid the phagocytosis by macrophages and promote the uptake by cancer cell, suggesting the NPs could be promising candidates to treat colorectal cancer.	Cheng et al., (2019)
Human colon cancer cell lines (Caco-2, HT-29 and DLD-1) (*In vitro*)	72 h	200 μM CLA	Cell proliferation of human colon cancer cell lines was inhibited by all the isomers of conjugated linoleic acid. The maximum inhibition was shown by t9,t11-CLA, followed by t10,c12-CLA, c9,c11-CLA and c9,t11-CLA, respectively. The extent of apoptosis was also shown by all the isomers, maximum by t9,t11-CLA. The other isomers exhibited much lower apoptosis effect on Caco-2 cells.	Beppu et al., (2006)
Mice and colon cancer cell lines (*in vitro*)	4 weeks for *in vivo*	0.1% c9, t11 or t 10,c 12 CLA	*In vitro*: c9,t11 CLA significantly inhibited cancerous cell growth ($P<0.05$), whereas t10,c12 CLA isomer had no effect on cell migration. *In vivo:* c9,t11 CLA isomer significantly ($P<0.05$) inhibited the	Soel et al., (2007)

			activity of matrix metalloproteinases (MMPs), which are actively involved and generated in angiogenesis and metastasis. However, t10,c12 CLA isomer did not inhibit the activity of MMPs. Furthermore, both the isomers were equally effective in inhibiting the colon cancer cell metastasis *in vivo*.	
Colon cancer cells (i*n vitro*)	72 h	25 and 50 μM CLA	Data suggested that t10,c12 CLA exhibited cytotoxic effect through reactive oxygen species (ROS) generation and a subsequent endoplasmic reticulum stress-dependent apoptosis in colon cancer cells. Overall, t10,c12 CLA showed an inhibition effect on the proliferation of colon cancer cells. It was also included that t10,c12 CLA was more efficient pro-apoptotic fatty acid than c9,t11 CLA.	Pierre et al., (2013)
MCF-7 breast cancer cells (*in vitro*)	24 h	50 μM CLA	Data exhibited that t9, t11 was the most efficient CLA isomer which decreased the proliferation and migration, and induced the apoptosis of MCF-7 breast cancer cells after 24 h of treatment. Another important observation was that the t9, t11 CLA treatment reduced the intracellular and membrane-associated cholesterol levels.	El Roz et al., (2013)
Human colorectal adenocarcinoma cells (*in vitro*)	24 h	25, 50 and 100 μM CLA	Results revealed that t10, c12-CLA induced the expression of ATF3 mRNA and luciferase activity (of ATF3 promoter), which are the main factors associated with apoptosis in colorectal cancer. Overall, it was suggested that t10, c12 CLA isomer treatment can induce the apoptosis of colon cancer cells, but not the most common CLA isomer i.e. c9, t11 as reported by Lee et al., (2006) in a similar study.	Kim et al., (2015)

Table 3. Major findings of clinical and animal studies based on anti-inflammatory and immunomodulatory activities of CLA

Subject	Duration	CLA concentration in diet	Major findings	Reference
Mouse and *in vitro*	NR	-	Decreased pro-inflammatory cytokines and inhibited leukocyte recruitment in vivo. While, *in vitro*, attenuated NF-κB-dependent gene expression, decrease pro-inflammatory cytokine production and up-regulated Nrf2-regulated proteins	Villacorta et al., (2018)
In vitro (bovine mammary epithelial cells: BME-UV1)	-	-	Bovine mammary epithelial cells treated with CLA showed significantly lower levels of ROS when compared with other cells treated with ALA, γ-linolenic acid or linoleic acid. Reduced gene expression was observed for the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-10). Whereas, greater expression was also observed for PPAR- γ (anti-inflammatory).	Dipasquale et al., (2018)
Mice	4 weeks	0, 0.5, and 1.5% DHA in presence and absence of 0.5% CLA	Dietary CLA lowered EPA-, DHA-, and ALA-derived epoxides, PGF1α, PGF2α, and F2-isoprostanes. Overall, it was concluded that CLA elevated proinflammatory oxylipins, while DHA increased anti-inflammatory oxylipins and weakened the effect of CLA-induced pro-inflammatory oxylipins in adipose tissue.	Adkins et al., (2017)
Human patients with Crohn's disease (n=13)	12 weeks	6g/d (orally)	Orally CLA intake supressed peripheral blood T cells to produce pro-inflammatory cytokines (IFN-g, TNF-a and IL-17), reduced disease activity and increased the quality of life of patients with Crohn's disease. Oral administration of CLA did not show any negative effects and was well tolerated.	Bassaganya-Riera et al., (2012)
Human (n=90)	2 months	3 g/d	The level of high sensitivity C-reactive protein (hsCRP), IL-6 and malondialdehyde (MDA), which are the markers of inflammation and oxidative stress, decreased significantly from 7.48 to 5.95 mg/ml, 16.13 to 12.95 pg/ml and 3.7 to 2.4 mol/l, respectively in CLA group after 8 weeks. However, glutathione peroxidase (GPx) increased from 125±46.06 (week 0) to 171.4±68.90 (week 8)	Eftekhari et al., (2013)

			mmol/ml/min.	
Human (n=29) Healthy adults	8 weeks	20 g/day of butter enriched with CLA (1020±167 mg CLA/day)	Intake of CLA-enriched butter resulted in increased levels of anti-inflammatory IL-10 and reduced levels of pro inflammatory components (TNFα, IL-2, IL-8 and transcription factor NFκB). However, there was no significant effect on adiponectin, body composition, C-reactive protein, and IL-4 when compared to control group.	Penedo et al., (2013)
Human (n=10)	10 weeks	200 g/week of cheese enriched with CLA	Dietary supplementation of CLA enriched cheese exhibited significant ($p < 0.05$) reduction in pro-inflammatory markers such as IL-6 (4.58±0.94 vs. 8.08±1.57 pg/mL), IL-8 (28.59± 2.64 *vs.* 45.02±5.82 pg/mL), and TNF-α (32.09±17.42 *vs.* 53.58±25.67 pg/mL). Moreover, a substantial reduction was also observed in the extent of platelet aggregation (77.7±3.56 *vs.* 87.8±1.76%).	Sofi et al., (2010)
Mice	2 weeks	0.5- 1% CLA-supplemented diet	Data revealed that wound healing rate was significantly ($P<0.05$) faster in group fed 1% CLA supplemented diet as compared to control and/or group fed 0.5% CLA-diet. However, no significant difference was observed between the would healing rate between 0.5% CLA group and control. Overall, results showed that CLA supplementation reduced the levels of oxidative stress and inflammatory markers in experimental animals.	Park et al., (2010)
Human cells (Human THP-1 monocytes) (In vitro)	NR	NR	Suppressed levels of pro-inflammatory markers such as macrophage phenotype; reduced expression of cyclooxygenase (COX)-2 and cytosolic phospholipase-A2 (cPLA2) and monocyte chemoattractant protein-1 (MCP-1); reduced levels of prostaglandin E2 (PGE2) and matrix metalloprotease (MMP)-9. Overall, it was concluded that CLA reduce the inflammatory outputs of macrophages.	McClelland et al., (2010)
Mice	3 weeks	1% CLA supplemented diet	When colitis-induced mice were fed with CLA-based diet, a significant suppression was observed in weight loss, signs of colitis and inflammatory infiltration than that of control. Contrarily, when the mice with no colitis fed with CLA-based diet, a condition ‘steatosis’ was developed in which lipid metabolism	Moreira et al., (2018)

			by liver gets impaired resulting abnormal accumulation of fats in cells and organs. Results suggested that CLA is safe for use during gut inflammation but not at steady state conditions.	

Table 4. Major findings of clinical and animal studies based on cholesterol-lowering and anti-obesity effects of CLA

Subject	Duration of feeding	CLA Concentration in diet	Major findings	Reference
Cholesterol-lowering effects				
Male, female	9 weeks	20 g/day (oleic acid: CLA::80:20)	High intakes of an 80:20 mixture of cis-9, trans-11 and trans-10, cis-12 CLA raised the total- to HDL-cholesterol ratio in healthy volunteers.	Wander et al., 2010
Rats	6 weeks	0.5% trans-10, cis-12 CLA	Resveratrol and CLA significantly reduced body fat but did not do so when combined.	Arise et al.,2011
Rats	100 days	0.5% trans–trans CLA isomers	The trans–trans CLA-rich soy oil lowered the serum cholesterol and low-density lipoprotein–cholesterol levels by 41 and 50%, respectively, also lowered the liver lipid content and decreased the liver weight in the obese rats.	Gilbert et al.,2011
Rats	21 days	1% CLA and 63.2% of fructose	CLA in high-fructose diet, decreases serum LDL + VLDL and TG and plasma MDA concentrations as well as liver weight and liver cholesterol.	Kostogrys et al., 2010
Mice	4 weeks	0.4 g of CLS mixed with 1 kg of hyperlipidemic feed or 5 g every day	Decreased serum total cholesterol (TC), serum triacylglycerols (TAGs), serum low-density lipoprotein cholesterol (LDL-C), atherogenic index (AI), liver weight (LW), liver index (LI), liver TC, and TAGs of mice.	Li, R et al., 2010
Anti-obesity effects				
Children (6 to 10 year)	2 weeks	3 g/day (50:50 cis-9, trans-11 and trans-10, cis-12 isomers)	CLA supplementation decreased body fatness in 6–10 years old children who were overweight or obese	Racine et al., 2010

Mice	14 days	1% trans-10, cis-12-CLA	Trans-10, cis-12 isomer of conjugated linoleic acid (CLA) caused a rapid reduction of body and adipose mass in mice.	Ashwell et al., 2010
Male, female (human)	12 weeks	1.7 g CLA in 200 ml sterilized milk	CLA significantly decreased the body weight, body mass index, body fat mass, fat percentage, subcutaneous fat mass, and waist-to-hip ratio in overweight.	Chen et al., 2012
Rat	8 weeks	2% of the 50:50 (c-9, t-11: t-10, c-12) mixture of CLA and 2% phytosterols	Diet supplementations with CLA, phytosterols or their combination for 65 days were effective in reducing body fat, adipose tissue and feed consumption, and CLA, but not phytosterols, modulated the action of leptin in obesity.	Furlan et al., 2013