Conjugated Linoleic Acid: Chemical Structure, Sources and Biological Properties

Rahim AYDIN

Department of Animal Science, Faculty of Agriculture, Kahramanmaraş Sütçü Imam University, Kahramanmaraş - TURKEY

Abstract: Conjugated linoleic acid (CLA) is a group of geometrical and positional isomers of linoleic acid (C18:2, cis-9, cis-12). In contrast to linoleic acid, double bonds in CLA are usually located at positions 9 and 11 or 10 and 12 and each double bond can be either in the cis or trans configuration. Meat and dairy products from ruminant animals (such as milk, butter, yogurt and cheese) are the principal natural sources of CLA in the human diet. Egg and meat products from poultry contain less CLA than meat from ruminant animals (0.6 and 0.9 mg/g fat vs. 2.9 to 5.6 mg/g fat, respectively). Meat from turkeys contains much higher CLA relative to meat from chickens. Dietary CLA has been shown to have potent anticarcinogenic and antiatherogenic effects in animal models. CLA was also found to have a potent immune modulating activity characterized by increased blastogenesis and macrophage killing ability. In addition to these biological properties, CLA was reported to reduce body fat content and increase lean body mass in pigs and rodents. Because of these biological properties of CLA, recently there has been a lot of interest in enriching egg, meat and dairy products for human consumption.

Key Words: Conjugated linoleic acid, anticarcinogenic, antiatherogenic, fat reducer, immune enhancer

Konjuge Linoleik Asitin Kimyasal Yapısı, Kaynakları ve Biyolojik Özellikleri

Özet: Konjuge linoleik asit (CLA) esansiyel bir yağ asiti olan linoleik asitin (C18:2, cis-9, cis-12) pozisyonel ve yapısal izomer gurupları için kullanılan bir terimdir. Linoleik asitin yapısına kıyasla CLA içindeki konjuge olan çift bağlar, zincirde 9. karbon ve 11. karbon veya 10. karbon ve 12. karbonlarda yer almışlardır. CLA'nin yapısında her bir çift bağ cis veya trans konumunda yer alabilir. Ruminant hayvanlardan elde edilen et ve süt ürünleri (örneğin tereyağ, yoğurt ve peynir) insanların yiyeceklerinde bulunan doğal CLA kaynaklarıdır. Kanatlılardan elde edilen yumurta ve et (sırasıyla yaklaşık 0,6 ve 0,9 mg CLA/g yağ), ruminantlardan elde edilen ete kıyasla (2,9 ile 5,6 mg CLA/g yağ) CLA bakımından zengin kaynaklar değildir. Hindi eti tavuk etine kıyasla daha yüksek oranda CLA içerir. CLA'nin biyolojik özelliklerinden dolayı CLA'e büyük ilgi görülmektedir. Diyetle alınan CLA'nin hayvan modellerinde etkili antikarsinojen etkiye sahip olduğu ve kalp-damar hastalıkları riskini (antiaterojenik) düşürdüğü gösterilmiştir. CLA'nin bağışıklık sisteminde blastogenezisi ve makrofajların öldürme kabiliyetlerini artırdığı da bulunmuştur. Bu biyolojik aktivitelere ek olarak CLA'nin domuz ve kemiricilerin vücutlarındaki yağ oranını azalttığı da rapor edilmiştir. Bu biyolojik özelliklerinden dolayı; yumurta, kırmızı et ve süt ürünlerinin CLA ile zenginleştirilmesi insan beslenmesi bakımından önem kazanmıştır.

Anahtar Sözcükler: Konjuge linoleik asit, antikarsinojenik, antiaterojenik, yağ düşürücü, bağışıklığı artırıcı

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometrical isomers of linoleic acid (C18:2, cis-9, cis-12), an essential fatty acid for human and animals, and involves a double bond at positions 8 and 10, 9 and 11, and 10 and 12 or 11 and 13 (1). Each of these positional conjugated diene isomers can occur in cis-trans, trans-cis, cis-cis or trans-trans geometrical configurations.

For over 50 years, CLA has been known to be present in dairy products and other foods derived from ruminant animals (2). Interest in CLA increased substantially following the observation of anticarcinogenic effect in the animal models fed cancer stimulating compounds (i.e. 7, 12-dimethylbenz[a]antracene or DMBA). In addition to anticarcinogenic effects, CLA was also reported to inhibit atherosclerotic lesions, to increase immune function, to decrease body fat, and to increase lean body mass in several animal models. Since CLA is a mixture of several isomers, any isomer might have different activities or work synergistically. Currently, c-9, t-11 CLA and t-10, c-12 CLA isomers are the only isomers that have actually been shown to exert physiological properties (Figure 1).

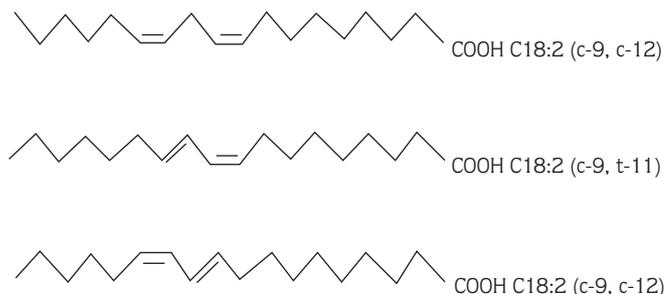Meat and dairy products from ruminant animals are

Figure 1. Structures of linoleic acid (top), c-9, t-11 CLA (middle) and t-10, c-12 CLA isomer (bottom).

the principal sources of CLA in the human diet. The concentration of CLA in cow's milk is variable and ranges from 2.4 to 21.8 mg of CLA/g of fat (3). The concentration of CLA in milk is known to vary among individuals and herds and between seasons. The greatest concentration of CLA was found during grazing period of animals. A total level of CLA in the human milk is lower than the cow milk and ranges from 0.37% to 0.75% of fat (4). The major CLA isomer in bovine (5) and human (4) milk was reported to be the c-9, t-11 CLA isomer. In meats and dairy products, the c-9, t-11 CLA isomer was shown to be the major CLA isomer and accounts for 75% and 90% of the conjugated dienes, respectively (2).

Biosynthesis of Conjugated Linoleic Acid

The major contributors to the formation of CLA in the foods are due to heat treatment (6) and microbial enzymatic reactions involving long chain fatty acids (mainly linoleic or linolenic acids) in the rumen (7). In the rumen, sequential reduction steps convert linoleic acid (C18:2 c-9, c-12) to the c-9, t-11 CLA, then to vaccenic acid (C18:1, t-11) and eventually to stearic acid (Figure 2) (8). CLA in the milk or meat from ruminant animals is derived from either CLA escaping from complete rumen biohydrogenation (9) or from absorbed C18:1, t-11, which is acted on by stearoyl-CoA reductase and converted to the c-9, t-11 CLA (7). Early studies showed that the level of CLA in the milk and butter was positively related to the intake of linoleic acid in diet (5).

Not only bacteria in the rumen, but also microorganisms in the digestive system of nonruminant animals (10) and humans (11) can synthesize CLA from long chain fatty acids. CLA was reported to be generated in the colon of conventional, but not in germ-free rats fed diets containing free linoleic acid (10).

Enhancement of CLA in Animal Products

Since dietary CLA has some biological properties, enhancing the CLA concentration of egg, milk and meat is of interest. Compared to meat and dairy products from ruminant animals, eggs and meat from poultry contain far less CLA (2). Laying hens fed a diet supplemented with 5% CLA for 29 days had enriched levels of CLA in egg yolks (12). However, dietary CLA was shown to cause adverse effects on the quality of eggs stored at 4 °C for 10 weeks (13,14). CLA in low-fat diets increased the level of saturated fatty acids (SFA) (mainly C16:0 and C18:0) and decreased the levels of monounsaturated fatty acids (MUFA) [mainly C16:1(n-7) and C18:1(n-9)] presumably due to an inhibitory effect of the t-10, c-12 CLA isomer on stearoyl-CoA desaturase enzyme. Enriching chicken eggs with CLA is possible by feeding CLA in the presence of any other fats (14). Chicken egg could be enriched 43-fold higher with CLA compared to control when 2% CLA and 4% canola oil were used together in chicken diet (15).

Research has demonstrated means of enriching the CLA content of animals and their products. A study showed that the concentration of CLA in the milk could be enhanced by the addition of sunflower oil (high in linoleic acid) and linseed oil (high in linolenic acid) in ruminant diets (16). Another study conducted in goat showed that 2% or 4% canola oil in the diet increased the levels of CLA 2- and 3-fold, respectively, in the milk compared to control (17). Others showed that feeding dairy cattle marine algae caused a 7-fold increase in the level of CLA in the milk compared to control (18).

The main isomers of CLA (i.e. c-9, t-11 CLA and t-10, c-12 CLA isomers) were shown to be incorporated into liver, heart, backfat, and omental fat of pigs fed CLA

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c-6, c-9, c-12              c-9, c-12               c-9, c-12, c-15
(gama linolenic acid)     (linolenic acid)        (alfa linolenic acid)
       |                        |                        |
       v                        v                        v
c-6, c-9, t-11             c-9, t-11               c-9, t-11, c-15
(conj. octadecatrienoic acid)  (conj. octadecadienoic acid)  (conj. octadecatrienoic acid)
       |                        |                        |
       v                        |                        v
c-6, t-11                       |                   t-11, c-15
(octadecadienoic acid)         |                   (octadecadienoic acid)
        \                       |                       /
         \                      v                      /
          ------->           t-11           <---------
                        (vaccenic acid)
                              |
                              v
                            C18:0
                        (stearic acid)
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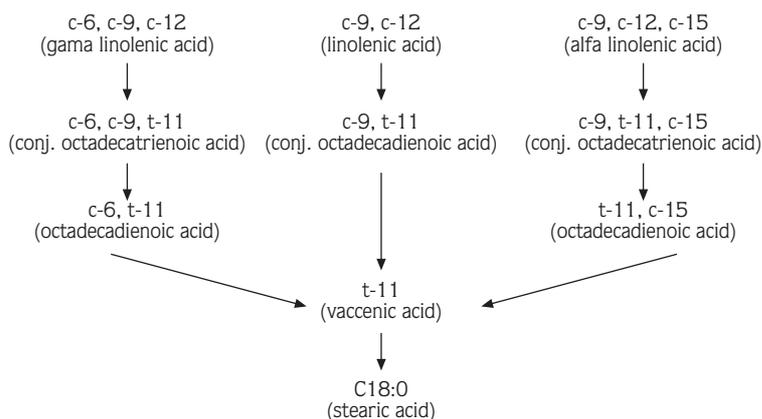
Figure 2. Predominant pathways of biohydrogenation of unsaturated C18 fatty acids (8).

mixture in a dose-dependent manner (19). The levels of the c-9, t-11 and t-10, c-12 CLA isomers decreased at similar rates in liver and fat pads when supplemental CLA was withdrawn from diet (20). The t-10, c-12 CLA isomer was reported to be cleared significantly faster than the c-9, t-11 CLA isomer in the skeletal muscle (20). It was suggested that the t-10, c-12 CLA isomer might be metabolized more rapidly than the c-9, t-11 CLA isomer (19), particularly in skeletal muscle (21).

Biological Properties of CLA

Anticarcinogenic Activities of CLA

The existence of CLA has been known for over 50 years. However, the recent interest in CLA began with the isolation from hamburger meat as an anticarcinogenic factor (22). Partially purified extracts from fried ground beef was shown to contain mutagenic modulator activity inhibiting the initiation of mouse epidermal carcinogenesis by 7, 12-dimethylbenz[a]antracene (DMBA), a procarcinogen (22). Then, this anticarcinogenic compound was isolated and identified, and designated as CLA (6).

In the 1990s, CLA was repeatedly shown to have anticarcinogenic effects in animal models for stomach neoplasia (23), mammary tumors (24), and skin papillomas (25). It was found that as low as 0.05% level of CLA was enough to significantly decrease the number of induced mammary tumors in rodents (26). CLA was shown to be effective in reducing the size and metastasis of transplanted human breast cancer cells and prostate cancer cells in severely compromised immunodeficient (SCID) mice (27). This property of CLA is in sharp contrast to the procancer activity associated with feeding linoleic acid (28). A number of studies showed that linoleic acid actually enhanced the development of mammary tumors in rodents (29). Recently, it was shown that linoleic acid increased prostate cancer (27). Also, CLA was reported to inhibit the proliferation of human malignant melanoma, colorectal and breast cancer cells in in-vitro studies (30).

An epidemiological study conducted in Finland showed a significant inverse relation between milk consumption and incidence of breast cancer in women in a 25-year reporting period (31). Recently, CLA-enriched butterfat was reported to alter mammary gland morphogenesis and to reduce the risk of cancer in rats (32).

Like other PUFA, CLA may modulate carcinogenesis by mechanisms affecting the separate stages of cancer development known as initiation, promotion, progression and/or regression. However, the actual mechanism by which CLA affects carcinogenesis has yet to be described. A study suggested that CLA might act by antioxidant mechanisms (23). Others suggested that CLA might act by inhibiting nucleotide synthesis (30) or inhibiting both DNA-adduct formation (33) and carcinogen activation, as opposed to direct interaction with the procarcinogen, scavenging of electrophiles or selective phase I detoxification pathways (34). Some proposed that the mechanism of tumor inhibition by dietary CLA might be related to its ability to regulate lipoxygenase and cyclooxygenase lipid mediators (35). Recently, it has been

hypothesized that there might be more than one mechanism by which CLA influences carcinogenesis (36).

B. Fat Reducing Activity of CLA

Generally, fatty acids containing trans double bonds have a negative impact on lipid metabolism and depress the amount of milk fat (37). While linoleic acid has two double bonds with cis configuration (C18:2 c-9, c-12), CLA has conjugated double bonds with possible cis and/or trans configurations (Figure 1).

Dietary CLA increased the level of total saturated fatty acids (mainly C14:0, C16:0 and C18:0) whereas that of monounsaturated fatty acids (mainly C18:1, n-9) was decreased (14). The shift toward a higher deposition of saturated fatty acids and lower deposition of monounsaturated fatty acids is the result of down regulation of stearoyl-CoA desaturase activity by t-10, c-12 CLA isomer (38). Changes in the body composition, such as reduced body fat, enhanced body protein, ash and water were found to be associated with the feeding the t-10, c-12 CLA isomer in mice, but not the c-9, t-11 CLA isomer (39). CLA was shown to alter the fatty acid composition of milk fat and inhibit milk fat secretion in dairy cows (40). In this study, the researchers showed that CLA content (% of fatty acid methyl esters) of milk fat increased in a dose-dependent manner. They also reported that CLA infusion into abomasum of cows had no effect on milk protein, but reduced the content and yield of milk fat (40). The complete mechanism by which dietary CLA causes decreased milk fat accumulation is not known.

Dietary CLA was shown to decrease subcutaneous fat depot and increase lean body mass in pigs fed a cereal-based diet containing 2% CLA compared to pigs fed control diet (2% sunflower oil) (41). Also Dugan et al. (41) reported that dietary CLA tended to improve feed efficiency in the pigs. Another study conducted in pigs confirmed those findings shown by Dugan et al. (42).

Studies conducted in dairy cows demonstrated that abomasal infusion of CLA decreased milk fat yield. Those effects may be due to CLA isomers containing a t-10 double bond (43). In another study, t-10, c-12 CLA was shown to result in a curvilinear reduction in milk fat ranging from 25% with abomasal infusion of 3.5 g/d to 50% with 14.0 g/d (44).

Conjugated linoleic acid (CLA) was shown to reduce body fat mass (BFM) in animals. To investigate the dose-response relationships of CLA with regard to BFM in humans, a randomized, double-blind study including 60 overweight or obese volunteers (body mass index 25–35 kg/m^2) was performed (45). The subjects were divided into five groups receiving placebo (9 g olive oil), 1.7, 3.4, 5.1 or 6.8 g CLA per day for 12 wk, respectively. In that study conducted in obese humans, CLA was shown to reduce body fat mass and that no additional effect on body fat mass was achieved with doses >3.4 g CLA/d (45). It was also found that the reduction of body fat within the groups was significant for the 3.4 and 6.8 g CLA groups (P=0.05 and P=0.02, respectively). No significant differences among the groups were observed related to lean body mass, body mass index, blood safety variables and blood lipids (45).

C. Antiatherogenic Activity of CLA

While considerable research has focused on a potential anticarcinogenic effect of CLA, there are few studies indicating that CLA may also reduce the risk of cardiovascular diseases in animal models (46,47). A study conducted in rabbits showed that dietary CLA resulted in a marked decline in the levels of total plasma cholesterol, triacylglycerol, and the ratio of LDL to HDL cholesterol (46). In addition, less atherosclerosis was detected in the aortas of the rabbits fed CLA relative to the control (46). Similar results on cholesterol metabolism were reported in hamsters fed CLA. The hamsters fed CLA had lower levels of total plasma cholesterol, non-HDL cholesterol (very low and LDL included), and triacylglycerol compared to control-fed hamsters. An antioxidant effect of CLA was suggested by determination of plasma tocopherol/total cholesterol ratios. Similar to the findings by Lee et al. (46), measurement of the aortic fatty streak areas revealed less early atherosclerosis in the CLA-fed hamsters (47).

Recently, a study conducted in hamsters showed that a mixture of CLA influenced body weight gain and plasma lipids (48). In this study, the three experimental diets fed to the hamsters consisted of the mild atherogenic diet plus CLA mixture at 10g/kg diet (CLA group), c-9, t-11 CLA at 2g/kg diet (c-9, t-11 group) or linoleic acid at 2g/kg diet (LA group). The CLA mixture was reported to decrease the levels of plasma triacylglycerol, total cholesterol and non-HDL cholesterol significantly after 2 weeks or 6 weeks of feeding the treatments compared to the LA group, but not the c-9, t-11 CLA group (48).

In the literature, dietary CLA has been reported to have controversial results on atherosclerosis in mice. One reported that CLA actually did not reduce the incidence of atherosclerosis, but increased the incidence of fatty acid streaks in mice compared to control-fed mice (49). The mice fed CLA had significantly lower concentration of serum triacylglycerol and a significantly higher ratio of serum HDL-cholesterol:total cholesterol (49). The addition of CLA to the atherogenic diet actually was shown to increase the development of aortic fatty streaks. This study conducted by Munday et al. (49) contradicts the finding of studies conducted in rabbits and hamsters (46,47). It is possible that dietary CLA has different influences on the fatty acid and cholesterol metabolism in different animal species. More research is necessary to elucidate the effects of dietary CLA on lipid metabolism and atherogenesis in animal models and eventually human beings.

The consequence of the inhibition of stearoyl-CoA desaturase by CLA may be relevant to lipoprotein metabolism (50). The secretion of VLDL from liver requires synthesis of an apoprotein called apoB-100 and sufficient amount of oleic acid (C18:1, n-9) (51). CLA was shown to decrease the levels of plasma triacylglycerol, VLDL and LDL in rabbits (46). It was also shown that the synthesis of apoB-100 and secretion of triacylglycerol in Hep G2 cells were inhibited by CLA (46). C18:1, n-9 stimulated both the synthesis and secretion of VLDL and apoB-100 in HepG2 and Caco-2 cells (52). The researchers suggested that increased secretion of apo-B in response to C18:1(n-9) could be due to increased de novo synthesis of apo-B (52). Mice fed for 2 weeks either a fat-free high carbohydrate diet or a 5% corn oil diet, supplemented with or without 0.5% CLA mixture (42% c-9, t-11 and 44% t-10, c-12 CLA isomers), had a 45% and 75% decrease, respectively, in stearoyl-CoA desaturase mRNA levels in the liver compared to control (38). Some suggested that CLA could result in a decrease in VLDL and apoB-100 secretion by inhibiting the stearoyl-CoA desaturase enzyme activity (50). The t-10, c-12 CLA isomer significantly reduced apolipoprotein secretion in the HepG2 cells compared to the linoleic acid control and the c-9, t-11 CLA isomer (53).

D. Role of CLA in Immune Function

Dietary CLA was shown to prevent immune-induced growth suppression following endotoxin (lipopolysaccharide, LPS) injection in chicks and rats (35). What was observed was that the control fed LPS injected chicks lost weight over 24 hours, but those fed CLA continued to grow even though immune stimulated. The study was repeated in mice and rats showing that CLA protected against immune stimulation across animal species (54).

Conclusion

CLA has been shown to have many biological activities (i.e. anticancer activity, immune-enhancing activity, weight-reducing effects and possible antiatherogenic properties) in animals at levels below 1% of the total energy in the diet (26). Because of those biological properties of CLA, recently there are a lot of interest in enriching egg, meat and dairy products for human consumption. It is possible to change the lipid composition of food products easily, such as eggs, milk or meat, by modifying the diet of the animals. Daily intake of CLA is not well documented, but has been estimated to be under several hundred mg/d (26). Animal data indicated that about 3.0 g/d of CLA may be necessary for beneficial effects in humans (26).

Since CLA is a mixture of several isomers, any isomer might have different activities or work synergistically. Currently, the c-9, t-11 CLA and t-10, c-12 CLA are the only isomers that have actually been shown to exert physiological properties. It is not known if the other CLA isomers have biological activities as well. As the individual CLA isomers become more available, studies have been conducted to compare the isomers of CLA and find out physiological activities of certain isomers.

Today, CLA has been studied extensively mainly in USA, France, Japan, Italy, and Norway. The studies conducted up to now produced more questions than answers. Especially the mechanisms how CLA works in health and disease conditions are not clear. Therefore, it appears it will take much longer to elucidate the function of CLA in health and diseases.

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