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Review Article

Functions and Health Benefits of Conjugated Linoleic Acid: A Review of Controlled Clinical Trials

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Abstract

Introduction: The term "Conjugated linoleic acid (CLA)" refers to a class of linoleic acid isomers. During the recent years, scientists have particularly regarded this fatty acid as a functional food. However, the findings of the studies in health benefits of CLA are inconsistent. The purpose of this study was to review the articles that investigated the effects of CLA on different aspects of health.
Materials and Methods: Controlled clinical trials including human, animal and in vitro studies having been published since 2000 till 2016 in English were searched in PubMed, ISI Web of Science, Scopus and Science Direct databases. Sixty four articles were finally selected to review the results.
Results: Findings of the researche indicated that CLA has an anticarcinogenic function. In some studies CLA had beneficial effects on bone metabolism and it is suggested that CLA may be useful in prevention and treatment of osteoporosis. In addition supplementation with CLA in animal and human models leaded to reduction in body fat mass and improvement in insulin resistance and lipid profile. However, some studies reported adverse effects of CLA intake including accumulation of fat in tissues, elevation of lipid peroxidation and inflammation markers.
Conclusion: Although benefits of CLA intake are shown in several studies, side effects of this fatty acid are reported in other researche. It is necessary to design further studies to more precise investigation of the benefits and risks of CLA.

Keywords: Conjugated linoleic acid, Health, review

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Introduction

The term "conjugated linoleic acid (CLA)" refers to a class of linoleic acid isomers that have conjugated double bonds (1). CLA belongs to the family of omega-6 fatty acids (1). The main dietary sources of this fatty acid are ruminant meat and dairy products (2). CLA can be produced via two distinct pathways in the body of ruminants: First, partial hydrogenation of linoleic acid in the rumen accomplished by the bacteria, and second, through the enzymatic dehydrogenation of vaccininc acid which normally happens in certain tissues (3). The active isomers of this 18 carbon fatty acid are 9-cis, 11-trans and 10-trans, 12-cis (3). The main isomer of CLA in foods is 9-cis, 11-trans (3). Finding CLA as a functional food dates back to 1979 (4) when Pariza and his colleagues found that ruminant meat contains potential anti carcinogens and consequently they could identify CLA isomers in meat (4). During the recent years, scientists have particularly regarded this fatty acid as a significant dietary compound. Many controlled clinical trials have studied CLA multiple effects on various aspects of the general health in both animal and human models. Numerous health benefits of CLA have been reported in these publications; however the results obtained still show inconsistency. For instance, Warren and his colleagues tried to discuss that in addition to reducing body fat mass, CLA can induce hepatic lipid accumulation and may exacerbate insulin resistance(5). The present study seeks to review the controlled clinical trials and all the relevant new data so far accomplished that have outlined in details the overall effects of CLA on the general health and disease and offered certain potential mechanisms of CLA actions pertaining to the nutritionally based therapeutic applications of CLA.

Materials and Methods

Search strategy and engines

To achieve the present study's objectives certain data bases as Google Scholar, ISI Web of Science, Science Direct, PubMed, Scopus, together with the key words " conjugated linoleic acid , clinical trial, supplementation, health benefits, body composition, diabetes, lipid profile, cancer, bone metabolism and also various other related words and phrases have comprehensively been searched since 2000 till 2016. Key words were selected through the MeSH database.

Inclusion and exclusion criteria

All of the controlled clinical trials including human, animal and in vitro studies having been published since 2000 till 2016 in English were enlisted in the study with no limitation regarding the CLA type or the number of isomers being applied in the controlled clinical trials. Articles having no available full-text and also due to duplication from multiple data bases were essentially excluded from the study. Sixty four articles were finally selected to start the clinical review efficiently.

Results and discussion

To ensure compliance of the articles having been searched with the inclusion and exclusion criteria, titles and the abstracts of the articles were critically assessed by two reviewers. From a total of 142 articles having the inclusion criteria, 43 articles due to duplication in multiple databases, 14 because of unavailability of the full-text, 10 articles for a questionable methodology and 11 due to the incompatibility of the text with the review objectives were excluded from the study. At the end of exclusion process, 64 articles were left and selected for the closer inspection and exploitation of the results, 28 of which had been performed on the human models, while 31 of them pertained to the animal models and 5 studies belonged to in vitro experiments. Article flow diagram has been shown in figure 1.

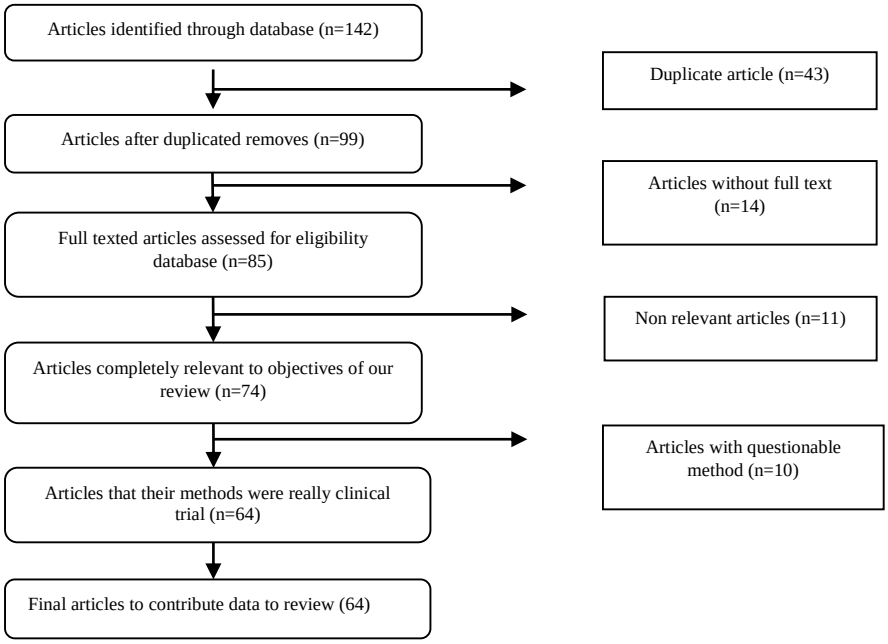

Figure1. Article flow diagram

CLA effects on weight and body composition

Many researches have closely inspected the effects of a CLA -enriched diet on the alterations of body weight and fat mass. In the study of Rahman et al. Dietary CLA supplement (containing equal amounts of two isomers 9-cis, 11-trans and 10-trans, 12-cis) in either of the two forms, free fatty acids and triglyceride, applied in two groups of obese rats led to significant loss of visceral fat among both groups of CLA recipients as compared to the control group. In another clinical trial being studied on the obese rats, only isomer 10-trans, 12-cis (and not isomers 9-cis, 11-trans) could cause fat mass decrease and induced apoptosis among the adipocytes (7). Warren et al. reported that 10-trans, 12-cis CLA isomer daily administration as much as about 5% of the total energy intake over 2-week duration could significantly induce 5% visceral fat loss among rats (5). Herein, no such specific effects pertaining to the isomer 9-cis, 11-trans were presented. A few more animal studies also claimed that isomer 10-trans, 12-cis of CLA is the one responsible for the fat mass reduction (8-10). However, some evidence suggest that body fat mass reduction has been associated with the fat accumulation and weight gain of some tissues as spleen, muscles and liver in return (5, 11, 12). In the other words this isomer is perceived to modify body fat distribution pattern in various body tissues.

In the animal studies, CLA effects on weight and fat mass loss is substantially a subordinate of the original race and weight of the animal. As among the fat rats of Zucker race CLA supplementations led to 15% peritoneal fat gain and among the same race rats with normal weights resulted in a 24% fat mass loss (13). It is believed that genetic inclination to fat accumulation among the obese rats of Zucker race, not only keeps existing but will intensify as well. On the other hand CLA supplementation among the obese rats of OLETF race induced a significant visceral fat reduction adversely (6).

As similar with the animal studies, multiple human researches have also presented abundant beneficial effects of dietary CLA supplementation on various body composition indexes (14-17). However, some of the human research studies demonstrate CLA supplementation failing to reduce body fat mass inconsistently (18-20). The fact underlying that seems to be CLA supplementation treatment at a lower dose per Kg body weight among human models as compared to the animals. Furthermore, since laboratory animals underwent physical examination throughout the growth period, so the type of response to the CLA supplementation could basically differ with the mature human model responses.

In a long-term clinical trial with a large sample size performed by Gaullier et al. on the healthy overweight and obese human models, the therapeutic effects of dietary CLA supplementation on body composition were studied (14). In the study mentioned, 118 participants received a daily 3.4 gr of dietary CLA supplements (equal amounts of both isomers) for duration of 6 months. At the end of

treatment the total body fat mass, localized fat tissue in the thigh region and the waste to hip ratio (WHR) underwent a significant decrease in the CLA recipients as compared to the placebo group. Among the CLA hosts, body free fat mass as well, indicated a significant increase remarkably. At the end of the study the lowered weight and body mass index (BMI) was only significant among those having BMI over 30 (obese participants) at the time being enlisted in the study, as compared to the placebo recipients. In a study by Chen et al. conducted on 33 obese and overweight human samples, the participants received 3.4 gr/day CLA isomers as a mixture with 400 cc milk for 12 week duration. At the end of the study, body weight, BMI, body fat mass, body fat percentage, subcutaneous fat mass and the WHR underwent a significant decrease comparing to the baseline values. The severity of decrease in all the variables except for the subcutaneous fat mass was more remarkable among women rather than men (15). In another controlled clinical trial accomplished on the 6-10 year old overweight and fat children, the recipients received 3gr CLA supplements (equal amounts of each isomer) for duration of 7 months via chocolate milk. By the end of the study CLA supplement recipients indicated a significant fall in the total body fat and abdominal fat percentage. Similar results were also obtained from the Carvalho et al. (17), Smedmann et al. (21) and Thom et al. (22) studies.

In a double blind randomized crossover design study in type 2 diabetic obese postmenopausal women carried out by Norris et al., 16 months supplementation with an isomer mixture of CLA supplementation by a dose of 6.4 gr/day exhibited a significant decrease in BMI and body fat mass as compared to the group of safflower oil recipients as placebo. However, the alteration in the free fat mass, WHR and skin fold thickness didn't appear to be statistically significant at the end (23).

Blankson et al. studied the probable correlation between the dietary CLA supplement dosage and the fat mass reduction among obese and overweight recipients (23). Participants received a mixture of two CLA isomers (equal amounts of each) as much as 1.7, 3.4, 5.1 and 6.8 gr per day for 12 week and then underwent body composition assessment. The two groups receiving 3.4 and 6.8 gr/day CLA supplements experienced significant shedding of body fat although exhibited no significant weight loss. Regarding the fact that the recipients of 6.8 gr dietary CLA supplements had considerably increased their physical activity during the study process, Blankson et al. recognized that a CLA dose of 3.4 gr per day would be sufficient for the body fat mass reduction (24).

Various other relevant studies have reported no significant effect of dietary CLA supplements on body composition. In a controlled clinical trial performed on the hyperchlosterolemic overweight men, supplementation with the two CLA isomers (equal amounts of each) and also by the 9-cis, 11-trans isomer as much as 3.5 gr per day for a duration of 8 weeks had caused no significant alteration in BMI, body fat mass and free fat mass as well (19). One of the remarkable limitations of this study has been the lack of supervision over the participants' diet quality during the treatment procedure. In the clinical trial accomplished by Deguire et al. CLA isomer 9-cis, 11-trans intake as much as 1.5 gr/day and 3 gr/day for a 16 week period among two groups of healthy men led to no significant alteration in BMI, WHR, body fat mass and free fat mass substantially (20). The results obtained from Shadman et al. study are also consistent with the Deguire's as well (25).

According to the reports presented by different publications, CLA intake especially 10-trans, 12-cis isomer, seems to leave some beneficial effects on various body composition indexes. Suggested mechanisms of CLA actions include contributing to increase in activity of fatty acid oxidation enzymes (1), stimulate apoptotic pathways in adipocytes and decrease in leptin gene expression (26). CLA also induces a lowered lipoprotein lipase enzyme activity and thereby inhibits the triglyceride removal by the adipocytes (19). Inconsistent results obtained from various studies could partially be the result of dietary CLA supplement dosage, isomers in use, treatment time duration and lack of uniform application of instruments and methods in the body composition assessment widely used in different studies.

CLA functions in type 2 diabetes

Type 2 diabetes has been known as one of the most widespread metabolic diseases throughout the world which involves insulin resistance or any malfunctions relevant to this hormone secretion (1). During the recent years many researchers have inspected some of the dietary functions of nutritional factors such as unsaturated fatty acids in the improvement of glycemic control among diabetic patients. In the study of Cho et al. dietary intake of CLA as much as 1% of the total energy intake (almost equal amounts of the two isomers) among male rats and for 8 week duration could significantly lead to a decrease in serum glucose and insulin levels and insulin resistance. Furthermore, glucose transporter 4 (GLUT 4) and peroxisome proliferator activated receptor-gamma (PPAR-γ) expression in the skeletal muscles of the same rats would also indicate a significant increase as well (27).

Ryder et al. enriched the rats' diet with a mixture of CLA isomers (1.5% of the total energy intake) and they presented a significant improvement in the glucose tolerance (28). In the researches performed by Zhou et al. (29) and Go et al. (30) a CLA enriched diet resulted in a significant decrease of the glucose and insulin concentration among rats. CLA could function as a PPAR-γ ligand and contribute to raising insulin sensitivity through the ligand activation (31). In this way CLA could be as equally effective as thiazolidinediones drugs that have beneficial effects for diabetic patients (31).

Although several animal studies have exhibited favorable effects of CLA supplements on the insulin resistance and glucose hemostasis, the results of the clinical trials on human models show multiple inconsistencies as well. These effects have partially originated from the certain isomer applied. Existing documents indicate that 10-trans, 12-cis isomer may leave negative effects on glycemic control (1, 18). Also it has been suggested that CLA effects on insulin resistance is perhaps self-achieved and independent from the effects that this fatty acid leaves on body composition (1). So that in the study of Riserus et al. daily intake of 3.4 gr 10-trans, 12-cis isomer of CLA for a 12 week duration

would lead to the elevation of serum glucose levels, HbA1c and insulin resistance among the participants suffering from metabolic syndrome though they reflected a significant decrease in the body fat mass and abdominal fat adversely (18). In the mentioned study, participants' diet and physical activity remained totally supervised and under control however, analysis adjusting for the possible confounding variables caused no considerable alterations in the significance of the results. In this study among the recipients of the CLA mixture of isomers no significant changes were observed regarding the body composition and glucose metabolism. Unfavorable effects of 10-trans, 12-cis CLA isomer on the insulin sensitivity may be widely modulated through the enhanced oxidative stress and the inflammatory conditions (32). Since the isomer mentioned before had induced significant raise in the renal secretion of 8-isoprostane-F2-α and serum levels of C-reactive protein (CRP) (32). Data obtained from multiple other studies are also supporting this mechanism of CLA action (19, 33). In another clinical trial performed by Shadman et al. on the diabetic patients, dietary CLA supplementation as much as 3gr/day (equal amounts of the two isomers) for 8 weeks could not stimulate a significant alteration on the serum glucose levels and HbA1c considerably (25). Norris et al (23) and Gaullier et al. (14) did also report the lack of a significant change in the serum glucose and insulin levels and insulin resistance following the CLA supplementation among the diabetic patients. In the study accomplished by Moloney et al. daily intake of CLA supplement (equal amounts of two isomers) as much as 3 gr and for 8 weeks resulted in an insulin sensitivity reduction among the type 2 diabetic patients accordingly (34). In another study dietary CLA supplementation as much as 4gr/day (equal amounts of each isomer) for a 12 week period induced an increase in the serum glucose and insulin levels and decrease in insulin sensitivity among the obese participants (35). It is worth remarking that the mentioned study didn't include a control group while included a very small sample size (9 participants) markedly (35). On the other hand Eyjolfson et al.in his study indicated that dietary intake of CLA as much as 4gr per day (to equal amounts of the two isomers) for 8 weeks led to increasing insulin sensitivity index and decreasing serum insulin levels among the young and healthy participants (36). Carvalo et al. also reported that a diet being enriched with a mixture of CLA isomers (equal amounts of two isomers) as much as 3 gr per day for 3 months induced significant reduction in the serum insulin levels at the end of the study as compared to the beginning stage (17). However the alterations in the serum glucose levels and insulin resistance were not significant markedly. Having a small sample size (17 participants) rooted from multiple inclusion and exclusion criteria are some of this study's limitations essentially.

Inconsistent results have caused abundant concerns regarding the safety and security of the dietary CLA supplements among diabetes patients. Therefore, complementary studies in this regard seem to be substantially required.

CLA effects on lipid profile

Increasing serum lipid levels has long been considered as one of the cardiovascular diseases risk factors (1). Lee et al. in his study indicated that daily administration of 5.1 g CLA (equal amounts of each isomer) for a period of 12 weeks could reduce total cholesterol levels, LDL cholesterol and triglyceride among the rabbits who were recipients of cholesterol enriched diet (37). Gavino et al. also showed that dietary intake of CLA isomers as much as 1% of the total energy intake for 6 weeks could cause reduction in the total cholesterol levels and triglyceride among rats (38). Plenty of other animal studies are supporting the present findings (39, 40). The health effects of dietary CLA supplements in lowering cholesterol levels is not still clearly defined. Noone et al. have anyhow reported lipid profile improvement following CLA intake among healthy participants (41). In this study 3 gr CLA intake per day (equal amounts of each isomer) for 8 weeks led to significant reduction in serum triglyceride levels. on the other hand the recipients of the same CLA supplement dose with a mixture of 80% of 9-cis, 11-trans isomer and 20% of 10-trans, 12-cis isomer could induce significant decrease in the VLDL levels essentially. Mechanisms through which CLA applies its favorable effects on the serum lipid levels are not so far properly defined. It has been suggested that CLA may cause reduction in the apolipoprotein B secretion involved in LDL cholesterol formation and increase of LDL receptor activity (31). Also this fatty acid lowers the cholesterol absorption from food via decelerating the intestinal enzyme sterol O-Acyltransferase expression (31). Blankson et al. in his study presented that CLA supplementation to 1.7, 3.4, 5.1 and 6.8 gr/day for 12 week duration among the obese and overweight participants led to significant decrease in HDL cholesterol among all the four groups (recipients of four supplement dosages) (24). Moreover, total cholesterol and LDL cholesterol levels among the recipients of 1.7, 3.4 gr/day CLA supplements showed a significant decrease accordingly. In the study of Joseph et al. dietary intake of only 9-cis,11-trans isomer and also a mixture of the CLA isomers failed to induce any significant alteration on any of the serum lipids among obese and hyperlipidemic men (19). Carvalho et al. (17), Tricon et al. (42) and Chen et al. (15) did report the lack of significant change in the serum lipids following a CLA enriched diet among the women suffering from metabolic syndrome, healthy men and obese and overweight men and women. In another controlled clinical trial CLA supplementation as much as 3.4 gr/day and for 6 weeks resulted in significant reduction in HDL cholesterol levels among obese and overweight participants. This happened while the average of other lipids levels remained drastically unchanged (14). Two other researches also reported the HDL cholesterol levels decrease following dietary CLA intake in human studies (16, 18).

According to the clinical trials being performed on humans, it is perceivable that CLA supplements are able to lower HDL cholesterol levels that functions as a protection against cardiovascular diseases, however, fails to apply any significant changes in the other lipids levels. On the other hand, in two different studies being designed by Basu et al. dietary CLA intake for 1 and 3 months induced increase in

the enzymatic and non enzymatic lipid peroxidation among healthy participants (44, 43). In another clinical trial by Smedman et al. carried out on the obese and healthy participants, 10-trans, 12-cis isomer could increase 8-isoprostaneF2α (the nonenzymatic lipid peroxidation index) obviously (45). Various other researchers have documented the same results as well (32, 46). CLA is abundantly potent for being oxidized due to including multiple double bonds in its chemical structure which could endanger the overall health condition of the recipients receiving high doses of this fatty acid (43). As a general conclusion beneficial health effects of CLA intake on blood lipid profile improvements is not well evidenced yet and still needs more studies targeting the issue in order to establish certain accurate findings.

CLA functions in cancer prevention

Although various evidences obtained from epidemiological studies argue the correlation between dietary fat intake and incidence of some cancers (47, 48). During the recent years it has been exhibited that some of the fatty acids as CLA could serve as cancer prevention in some of the cancers like breast, prostate and gastrointestinal efficiently (49, 50). Many studies that have specifically focused on the CLA health effects on cancer prevention and treatment are limited to animal models and in vitro studies while the clinical trials being performed on the cancer patients are noticeably rare. In the study done by Yang et al. rats were fed by a CLA isomers mixture enriched diet as much as 0.5% of the total energy intake and then a week following CLA treatment they were exposed to a carcinogenic chemical known as PHPI over a period of 61 days (49). According to the evidence obtained from this study, CLA could stimulate reduction in the colon cancer incidence among rats to 14% as compared with the control group. Park et al. in his study demonstrated that dietary CLA administration to 1% of the total energy intake could lead to a significant decrease in the 1,2-dimethyl hydrazine induced colon tumors among rats (50). In another clinical trial performed on the patients suffering from rectal cancer, the participants prior to the beginning of the radiotherapy and during the therapy as well, received CLA isomer mixture as much as 3 gr per day (51). By the study being finalized the serum level of inflammatory factors such as Tumor necrosis factor-α (TNF- α), Interleukin-1β (IL-1β) and CRP also the enzyme matrix metalloproteinase (MMP) levels which stands as angiogenesis and tumor spread marker indicated significant reduction among the group of CLA recipients. Among some of the animal models, CLA administration as much as 0.1% to 1% of the total energy intake demonstrated beneficial health effects on the growth suppression of the breast tumors and reducing mortality rate originating from this cancer while no favorable health effects have yet been reported applying higher doses of this fatty acid remarkably (52, 53). In the study of McGowan et al. CLA supplementation as much as daily intake of 7.5 gr isomer mixture over a 20 days period prior to the surgery among the breast cancer patients stimulated reduction in the protein Ki-67 expression (tumor proliferation marker) and S14 (nuclear modulator of the lipid synthesis (54).

It has been suggested that CLA applies its anti-cancer function through PPAR-γ (55-56). Synthetic PPAR-γ agonists in animal models have proved to serve as anti-cancer agents against potential carcinogens and prevent cancerous tumors metastasis extensively (56). In the study of Ochoa et al. carried out on the prostate cancer cell lines, CLA treatment suppressed cell proliferation, reduced eicosanoids synthesis and induced apoptosis through modulation of protein p53 expression (57). In an in vitro study both CLA isomer caused suppressed proliferation in the prostate cancer cells (56). IP et al. showed that CLA supplements can cause angiogenesis inhibition and reduces blood supply to the tumor cells in breast cancer (59). This mechanism of CLA action has great importance in tumor growth suppression in variety of cancers (59). Furthermore it has been argued that dietary CLA may prevent cancer cachexia through suppression of pre-inflammatory cytokines formation (1).

As a general conclusion both of the CLA isomers exhibit inhibitory effects against some cancers as colon, breast and prostate cancers and the majority of the studies focusing this issue are basically consistent.

Protective role of CLA in osteoporosis

Osteoporosis is a kind of disease that involves extensive bone loss and is considered as one of the most remarkable worldwide health disorders (60). Studies have shown that in addition to the CLA beneficial health functions in weight loss, this fatty acid never proved to leave any unfavorable effects on bone mass (60). Regarding the fact that osteoporosis is a common health condition during menopause and is the result of lowered secretion of ovarian hormones (60), Kelly et al. (61) meaning to create similar conditions, designed a research study upon the ovariectomized rats. In this study, rats were fed applying doses of 2.5, 5, and 10 gr CLA per kg body weight. At the end of the study, rats belonging to the second and third group, demonstrated significant decrease in the bone resorption markers such as Prostaglandin E2, Pyridinoline and Deoxypyridinoline (61).

In the study of park et al. dietary CLA as much as 0.5% of the total energy intake over a 4 weeks period stimulated bone mass increase among male rats (62). In another study performed by the same researcher upon the ovariectomized rats, CLA supplements could induce reduction in the Tartrate-resistant acid phosphatase levels in the femur bone which indicated suppressed osteoclastogenesis (63).

In the study of Rahman et al. bone mineral density among rats being fed by CLA 10-trans, 12-cis isomer enriched diet over a period of 6 months was significantly higher than the group of 9-cis, 11-trans isomer recipients and the control group (64). Also, the osteoclastogenesis factors such as TNF-α, IL-6, RANKL and the number of adipocytes in the bone marrow tissue underwent a significant decrease in this group. Other researchers have also suggested that CLA beneficial effects on bone metabolism are abundantly the result of 10-trans, 12-cis isomer function (62, 65). The results obtained from various other animal studies are confirming the favorable health effects of dietary CLA in preventing bone resorption as well (53, 64). The present evidences are indicating that CLA supplements could

accelerate bone mineralization and decelerate bone resorption due to suppressing PGE2, increasing calcium absorption, inhibiting inflammatory processes and lowering leptin levels (60). CLA could increase the expression of the proteins involved during the bone formation process as osteocalcin and alkaline phosphatase (64). In addition to that this fatty acid can stimulate osteoblastogenesis and inhibit adipogenesis respectively through metabolic pathways regulated by PPAR- γ (67). Nevertheless animal studies have indicated that CLA effects on bone formation may be influenced by the type of dietary fat, the balance existing in the structure of polyunsaturated dietary fatty acids and also the growth stage of the lab animal (60). The numbers of the clinical trials focusing specifically on the CLA function in human bone metabolism are noticeably rare. These publications have generally reported the lack of effect of this fatty acid supplementation on the bone mass and the bone formation or resorption indexes. However, CLA does not appear to leave any unfavorable and undesirable effect on human bone metabolism. In the study of Kreider et al. short term CLA supplementation (28 days) as much as 6gr/day among athlete men stimulated bone mass augmentation while this was not regarded as a significant increase (68). In a study done on human monocytes, CLA 9-cis, 11-trans isomer could significantly inhibit osteoclastogenesis (69). Also in this study both CLA isomers reduced osteoclasts activities remarkably. DeGuire et al. (19) reported that 9-cis, 11-trans isomer as much as 1.5 gr/day and 3gr/day over a 16 week period among healthy men didn't induce significant alteration in BMD and parathormone levels. In another clinical trial that involved 1 year treatment, the obese and healthy men were divided in 3 groups, recipients of 3gr/day CLA in the forms of fatty acid, triglyceride and placebo. As the study was finalized BMD alterations among each group were substantially non-significant (70). According to the favorable results obtained from multiple studies on animal models and human cell lines, researchers suggest that dietary CLA intake may serve beneficially in both prevention and treatment of various human bone diseases such as osteoporosis and rheumatoid Arthritis equally (60).

Other CLA functions

Te results acquired from studies investigating CLA effects on inflammatory processes are extensively divergent as some of them have reported suppression of pre-inflammatory cytokines such as TNF-a (32, 44, 71) while some other reported no considerable change or increase in the inflammatory factor levels like CRP in reverse (14, 19, 46, 72, 73). Yang et al. believed that 9-cis, 11-trans isomer is specifically responsible for the lowered TNF-a synthesis (74). Also CLA supplement, is able to reduce prostaglandin synthesis through minimum exposure to arachidonic acid and inhibiting the enzyme cyclooxygenase as well (1).

CLA supplementation argued in some studies could noticeably lower the appetite and energy intake (2, 14). It has been discussed that CLA could well affect protein expressions (14, 61, 75) involved in the appetite control such as neuropeptide-Y and leptin (6, 14, 75). Other CLA functions could be regarded as moderating hypertension and lowering the risk of cardiovascular diseases that have broadly been discussed in many studies (76).

Conclusion

According to the evidences achieved from the present review, CLA is believed to function as a protective agent towards cancers like colon, breast and prostate cancers. It could be suggested that this fatty acid applies favorable effects on bone metabolism and can serve as a preventive and therapeutic factor for osteoporosis accordingly. Furthermore many publications have recommended CLA supplements for body fat mass and weight loss, insulin resistance status and lipid profile improvements. Nevertheless, some evidences have presented CLA supplementation health risks inconsistently. Apparently, 10-trans, 12-cis CLA isomer may deteriorate insulin resistance. The evidences obtained from various clinical trials demonstrate lipid peroxidation and inflammation factors augmentation following CLA supplement intake. Future studies on health benefits and risks of various CLA isomers may be outlined to provide comprehensive explanations for the existing ambiguities and exhibit certain recommendations for the safe and secure intake of this fatty acid.

References

1. Wahle KW, Heys SD, RotondoD. Conjugated linoleic acids: are they beneficial or detrimental to health? Prog Lipid Res. 2004;43(6):553-87.
2. Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS. Effect of conjugated linoleic acid supplementation after weight loss on appetite and food intake in overweight subjects. Eur J Clin Nutr. 2003;57(10):1268-74.
3. Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem. 2006;17(12):789-810.
4. Pariza MW, Ashoor SH, Chu FS, Lund DB. Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Lett 1979;7:63-9.
5. Warren JM, Simon VA, Bartolini G, Erickson KL, Mackey BE, Kelley DS. Trans-10,cis-12 CLA increases liver and decreases adipose tissue lipids in mice: possible roles of specific lipid metabolism genes. Lipids. 2003;38(5):497-504.
6. Rahman SM, Wang Y, Yotsumoto H, Cha J, Han S, Inoue S, et al. Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and beta-oxidation of fatty acid in OLETF rats. Nutrition. 2001;17(5):385-90.
7. Hargrave KM, Li C, Meyer BJ, Kachman SD, Hartzell DL, Della-Fera MA, et al. Adipose depletion and apoptosis induced by trans-10, cis-12 conjugated linoleic acid in mice. Obes Res. 2002;10(12):1284–90.
8. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids. 1999;34(3):235-41.
9. Halade GV, Rahman MM, Fernandes G. Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice. J Nutr Biochem. 2010;21(4):332-7.
10. Terpstra AH, Beynen AC, Everts H, Kocsis S, Katan MB, Zock PL. The decrease in body fat in mice fed conjugated linoleic acid is due to increases in energy expenditure and energy loss in the excreta. J Nutr. 2002;132(5):940-5.
11. Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, et al. Conjugated linoleic acid

supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes. 2000;49(9):1534-42.
12. West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am J Physiol.1998;275:R667-72.
13. Sisk MB, Hausman DB, Martin RJ, Azain MJ. Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. J Nutr. 2001;131(6):1668-74.
14. Gaullier JM, Halse J, Høivik HO, Høye K, Syvertsen C, Nurminiemi M, et al. Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. Br J Nutr. 2007;97(3):550-60.
15. Chen SC, Lin YH, Huang HP, Hsu WL, Houng JY, Huang CK. Effect of conjugated linoleic acid supplementation on weight loss and body fat composition in a Chinese population. Nutrition. 2012;28(5):559-65.
16. Racine NM, Watras AC, Carrel AL, Allen DB, McVean JJ, Clark RR, et al. Effect of conjugated linoleic acid on body fat accretion in overweight or obese children. Am J Clin Nutr. 2010;91(5):1157-64.
17. Carvalho RF, Uehara SK, Rosa G. Microencapsulated conjugated linoleic acid associated with hypocaloric diet reduces body fat in sedentary women with metabolic syndrome. Vasc Health Risk Manag. 2012;8:661-7.
18. Risérus U, Basu S, Jovinge S, Fredrikson GN, Arnlöv J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation. 2002;106(15):1925-9.
19. Joseph SV, Jacques H, Plourde M, Mitchell PL, McLeod RS, Jones PJ. Conjugated linoleic acid supplementation for 8 weeks does not affect body composition, lipid profile, or safety biomarkers in overweight, hyperlipidemic men. J Nutr. 2011;141(7):1286-91.
20. Deguire JR, Makarem N, Vanstone CA, Morin S, Duque G, Weiler HA. Conjugated linoleic acid is related to bone mineral density but does not affect parathyroid hormone in men. Nutr Res. 2012;32(12):911-20.
21. Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans--metabolic effects. Lipids. 2001;36(8):773-81.
22. Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat in healthy exercising humans. J Int Med Res. 2001;29(5):392-6.
23. Norris LE, Collene AL, Asp ML, Hsu JC, Liu LF, Richardson JR, et al. Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. Am J Clin Nutr. 2009;90(3):468-76.
24. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J Nutr. 2000;130(12):2943-8.
25. Shadman Z, Taleban FA, Saadat N, Hedayati M. Effect of conjugated linoleic acid and vitamin E on glycemic control, body composition, and inflammatory markers in overweight type2 diabetics. J Diabetes Metab Disord. 2013;12(1):42.
26. Kelley DS, Erickson KL. Modulation of body composition and immune cell functions by conjugated linoleic acid in humans and animal models: benefits vs. risks. Lipids. 2003;38(4):377-86.
27. Cho K, Song Y1, Kwon D. Conjugated linoleic acid supplementation enhances insulin sensitivity and peroxisome proliferator-activated receptor gamma and glucose transporter type 4 protein expression in the skeletal muscles of rats during endurance exercise. Iran J Basic Med Sci. 2016;19(1):20-7.
28. Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatsiaris T, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes. 2001;50(5):1149-57.
29. Zhou XR, Sun CH, Liu JR, Zhao D. Dietary conjugated linoleic acid increases PPAR gamma gene expression in adipose tissue of obese rat, and improves insulin resistance. Growth Horm IGF Res. 2008;18(5):361-8.
30. Go G, Wu G, Silvey DT, Choi S, Li X, Smith SB. Lipid metabolism in pigs fed supplemental conjugated linoleic acid and/or dietary arginine. Amino Acids. 2012;43(4):1713-26.
31. Aminot-Gilchrist DV, Anderson HD. Insulin resistance-associated cardiovascular disease: potential benefits of conjugated linoleic acid. Am J Clin Nutr. 2004;79(6 Suppl):1159S-1163S.
32. Risérus U, Arner P, Brismar K, Vessby B. Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care. 2002;25(9):1516-21.
33. Poirier H, Shapiro JS, Kim RJ, Lazar MA. Nutritional supplementation with trans-10, cis-12-conjugated linoleic acid induces inflammation of white adipose tissue. Diabetes. 2006;55(6):1634-41.
34. Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr. 2004;80(4):887-95.
35. Thrush AB, Chabowski A, Heigenhauser GJ, McBride BW, Or-Rashid M, Dyck DJ. Conjugated linoleic acid increases skeletal muscle ceramide content and decreases insulin sensitivity in overweight, non-diabetic humans. Appl Physiol Nutr Metab. 2007;32(3):372-82.
36. Eyjolfson V, Spriet LL, Dyck DJ. Conjugated linoleic acid improves insulin sensitivity in young, sedentary humans. Med Sci Sports Exerc. 2004;36(5):814-20.
37. Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis. 1994;108(1):19-25.
38. Gavino VC, Gavino G, Leblanc MJ, Tuchweber B. An isomeric mixture of conjugated linoleic acids but not pure cis-9, trans-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. J Nutr. 2000;130(1):27-9.
39. Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS. The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. Int J Obes Relat Metab Disord. 2003;27(7):840-7.
40. Thomas Yeung CH, Yang L, Huang Y, Wang J, Chen ZY. Dietary conjugated linoleic acid mixture affects the activity of intestinal acyl coenzyme A: cholesterol acyltransferase in hamsters. Br J Nutr. 2000;84(6):935-41.
41. Noone EJ, Roche HM, Nugent AP, Gibney MJ. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. Br J Nutr. 2002;88(3):243-51.
42. Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, et al. Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am J Clin Nutr. 2006;83(4):744-53.
43. Basu S, Risérus U, Turpeinen A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. Clin Sci (Lond). 2000;99(6):511-6.
44. Basu S, Smedman A, Vessby B. Conjugated linoleic acid induces lipid peroxidation in humans. FEBS Lett. 2000;468(1):33-6.
45. Smedman A, Vessby B, Basu S. Isomer-specific effects of conjugated linoleic acid on lipid peroxidation in humans: regulation by alpha-tocopherol and cyclo-oxygenase-2 inhibitor. Clin Sci (Lond). 2004;106(1):67-73.

46. Raff M, Tholstrup T, Basu S, Nonboe P, Sørensen MT, Straarup EM. A diet rich in conjugated linoleic acid and butter increases lipid peroxidation but does not affect atherosclerotic, inflammatory, or diabetic risk markers in healthy young men. J Nutr. 2008;138(3):509-14.
47. Hunter DJ, Spiegelman D, Adami HO, Beeson L, van den Brandt PA, Folsom AR, et al. Cohort studies of fat intake and the risk of breast cancer--a pooled analysis. N Engl J Med. 1996;334(6):356-61.
48. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res. 1994;54(9):2390-7.
49. Yang H, Stuart GR, Glickman BW, de Boer JG. Modulation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mutation in the cecum and colon of big blue rats by conjugated linoleic acid and 1,2-dithiole-3-thione. Nutr Cancer. 2001;39(2):259-66.
50. Park HS, Ryu JH, Ha YL, Park JH. Dietary conjugated linoleic acid (CLA) induces apoptosis of colonic mucosa in 1,2-dimethylhydrazine-treated rats: a possible mechanism of the anticarcinogenic effect by CLA. Br J Nutr. 2001;86(5):549-55.
51. Mohammadzadeh M, Faramarzi E, Mahdavi R, Nasirimotlagh B, Asghari Jafarabadi M. Effect of Conjugated Linoleic Acid Supplementation on Inflammatory Factors and Matrix Metalloproteinase Enzymes in Rectal Cancer Patients Undergoing Chemoradiotherapy. Integr Cancer Ther. 2013;12(6):496-502.
52. Amarù DL, Biondo PD, Field CJ. The Role of Conjugated Linoleic Acid in Breast Cancer Growth and Development. Open Nutraceuticals J. 2010;3:30-46.
53. Białek A, Tokarz A, Zagrodzki P. Conjugated linoleic acids (CLA) decrease the breast cancer risk in DMBA-TREATED rats. Acta Pol Pharm. 2016;73(2):315-27.
54. McGowan MM, Eisenberg BL, Lewis LD, Froehlich HM, Wells WA, Eastman A, et al. A proof of principle clinical trial to determine whether conjugated linoleic acid modulates the lipogenic pathway in human breast cancer tissue. Breast Cancer Res Treat. 2013;138(1):175-83.
55. Li D, Yang K, Li JS, Ke XY, Duan Y, Du R, et al. Antitumor efficacy of a novel CLA-PTX microemulsion against brain tumors: in vitro and in vivo findings. Int J Nanomedicine. 2012;7:6105-14.
56. Kuniyasu H, Yoshida K, Sasaki T, Sasahira T, Fujii K, Ohmori H. Conjugated linoleic acid inhibits peritoneal metastasis in human gastrointestinal cancer cells. Int J Cancer. 2006;118(3):571-6.
57. Ochoa JJ, Farquharson AJ, Grant I, Moffat LE, Heys SD, Wahle KW. Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for cis-9, trans-11 and trans-10, cis-12 isomers. Carcinogenesis. 2004;25(7):1185-91.
58. Palombo JD, Ganguly A, Bistrian BR, Menard MP. The antiproliferative effects of biologically active isomers of conjugated linoleic acid on human colorectal and prostatic cancer cells. Cancer Lett. 2002;177(2):163-72.
59. Ip C, Dong Y, Ip MM, Banni S, Carta G, Angioni E, Murru E, et al. Conjugated linoleic acid isomers and mammary cancer prevention. Nutr Cancer. 2002;43(1):52-8.
60. Watkins BA, Li Y, Lippman HE, Reinwald S, Seifert MF. A test of Ockham's razor: implications of conjugated linoleic acid in bone biology. Am J Clin Nutr. 2004;79(6 Suppl):1175S-1185S.
61. Kelly O, Cashman KD. The effect of conjugated linoleic acid on calcium absorption and bone metabolism and composition in adult ovariectomised rats. Prostaglandins Leukot Essent Fatty Acids. 2004;71(5):295-301.
62. Park Y, Terk M, Park Y. Interaction between dietary conjugated linoleic acid and calcium supplementation affecting bone and fat mass. J Bone Miner Metab. 2011;29(3):268-78.
63. Park Y, Kim J, Scrimgeour AG, Condlin ML, Kim D, Park Y. Conjugated linoleic acid and calcium co-supplementation improves bone health in ovariectomised mice. Food Chem. 2013;140(1-2):280-8.
64. Rahman MM, Halade GV, Williams PJ, Fernandes G. t10c12-CLA maintains higher bone mineral density during aging by modulating osteoclastogenesis and bone marrow adiposity. J Cell Physiol. 2011;226(9):2406-14.
65. Doyle L, Jewell C, Mullen A, Nugent AP, Roche HM, Cashman KD. Effect of dietary supplementation with conjugated linoleic acid on markers of calcium and bone metabolism in healthy adult men. Eur J Clin Nutr. 2005;59(3):432-40.
66. Thiel-Cooper RL, Parrish FC Jr, Sparks JC, Wiegand BR, Ewan RC. Conjugated linoleic acid changes swine performance and carcass composition. J Anim Sci. 2001;79(7):1821-8.
67. Kim J, Park Y, Lee SH, Park Y. trans-10,cis-12 conjugated linoleic acid promotes bone formation by inhibiting adipogenesis by peroxisome proliferator activated receptor-γ-dependent mechanisms and by directly enhancing osteoblastogenesis from bone marrow mesenchymal stem cells. J Nutr Biochem. 2013;24(4):672-9.
68. Kreider RB, Ferreira MP, Greenwood M, Wilson M, Almada AL. Effects of conjugated linoleic acid supplementation during resistance training on body composition, bone density, strength, and selected hematological markers. J Strength Cond Res. 2002;16(3):325-34.
69. Platt I, El-Sohemy A. Effects of 9cis,11trans and 10trans,12cis CLA on osteoclast formation and activity from human CD14+ monocytes. Lipids Health Dis. 2009;8:15.
70. Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H, et al. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. Am J Clin Nutr. 2004;79(6):1118-25.
71. Changhua L, Jindong Y, Defa L, Lidan Z, Shiyan Q, Jianjun X. Conjugated linoleic acid attenuates the production and gene expression of proinflammatory cytokines in weaned pigs challenged with lipopolysaccharide. J Nutr. 2005;135(2):239-44.
72. Ramakers JD, Plat J, Sébédio JL, Mensink RP. Effects of the individual isomers cis-9,trans-11 vs. trans-10,cis-12 of conjugated linoleic acid (CLA) on inflammation parameters in moderately overweight subjects with LDL-phenotype B. Lipids. 2005;40(9):909-18.
73. Steck SE, Chalecki AM, Miller P, Conway J, Austin GL, Hardin JW, et al. Conjugated linoleic acid supplementation for twelve weeks increases lean body mass in obese humans. J Nutr. 2007;137(5):1188-93.
74. Yang M, Cook ME. Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-alpha production, and modifies splenocyte cytokines production. Exp Biol Med (Maywood). 2003;228(1):51-8.
75. So MH, Tse IM, Li ET. Dietary fat concentration influences the effects of trans-10, cis-12 conjugated linoleic acid on temporal patterns of energy intake and hypothalamic expression of appetite-controlling genes in mice. J Nutr. 2009;139(1):145-51.
76. Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats. Biochem Biophys Res Commun. 2003;310(2):562-6.