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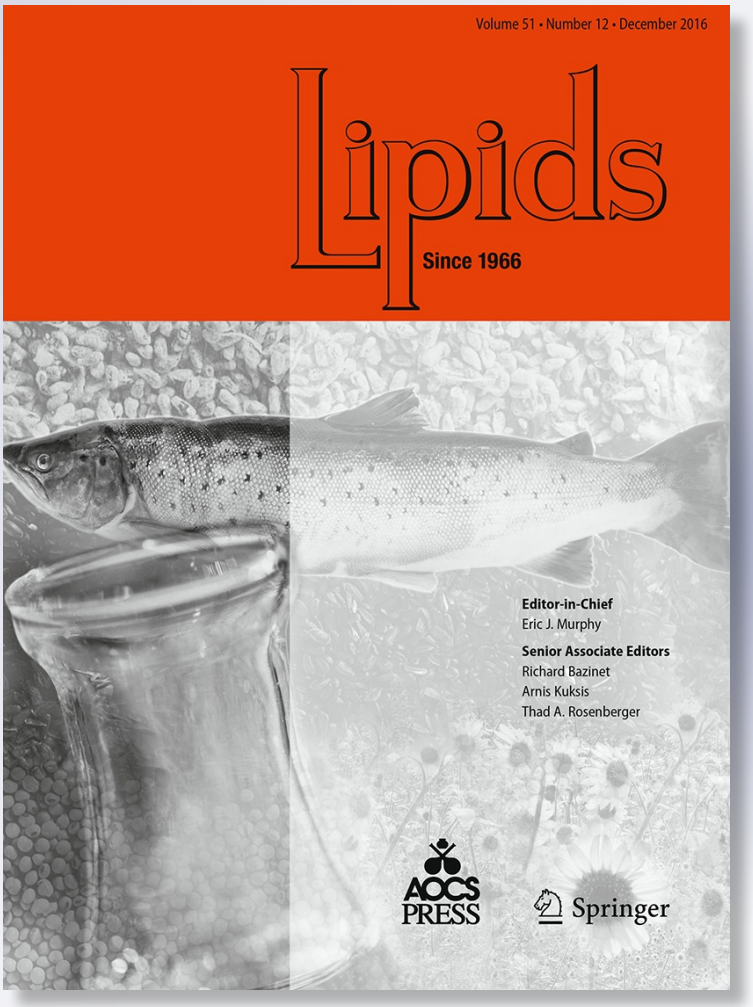

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ORIGINAL ARTICLE

CLA Has a Useful Effect on Bone Markers in Patients with Rheumatoid Arthritis

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Abstract Rheumatoid arthritis is a systemic, chronic disease which may increase the risk of osteoporosis. This study was carried out in order to examine the effect of conjugated linoleic acid (CLA) on bone markers in rheumatoid arthritis disease which is the most common autoimmune disease. The present study is a randomized double-blind clinical trial. Subjects included 52 patients with active rheumatoid arthritis who were divided into two groups. Group I received standard treatment plus 2 daily 1.25 g capsules (Containing about 2 g of 9-*cis* 11-*trans* isomer and 10-*cis* 12-*trans* isomer in ratio of 50 −50 CLA in glycerinated form), Group II received standard treatment plus 2 Placebo 1.25 g capsules containing sunflower oil with high oleic acid. Telopeptides C, osteocalcin, and MMP3 were analyzed by ELISA method, PGE_2 was done by competitive enzymatic immunoassay method, and IGF-1 was analyzed by the IRMA method based on the sandwich method and ALK-P of bone. Before and after the intervention, the questionnaires about general information, nutrition assessment and medical history were filled out by the subjects. Nutritional assessment was done by a 24-h record questionnaire for the three-day diet. The results were analyzed using SPSS software (version 18). Findings: There was no significant difference between the groups in enzyme activity of ALK-P of bone, PGE_2 and MMP3 variables. However, differences between the two groups in terms of activity of telopeptides C, Osteocalcin, and IGF1 were significant ($P < 0.05$). CLA has a potentially beneficial effect on bone markers in patients with rheumatoid arthritis. Therefore, in order to study the effect of CLA on bone health in patients with RA and all patients with autoimmune and bone diseases more studies with longer duration and evaluation of bone mass density are required.

Keywords CLA · Bone ALK-P · Telopeptide C · Osteocalcin · Rheumatoid arthritis

Abbreviations

ACR	American College of Rheumatology
ALK-P	Alkaline phosphatase
BMD	Bone mass density
BMI	Body mass index
BMP	Bone morphogenic proteins
CLA	Conjugated linoleic acid
FA	Fatty acid
IGF1	Insulin-like growth factor 1
MMP3	Matrix metalloproteinase 3
PGE_2	Prostaglandine E2
PPARγ	Peroxisome proliferator activated receptor gamma
RA	Rheumatoid Arthritis

Background

Rheumatoid arthritis is a systemic, autoimmune, and chronic disease [1]. The disease is in from of a synovitis that affects joints and leads to the destruction of cartilage

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and to bone lesions [2]. Both mediated cellular and humoral immune responses are involved in the development of this synovitis [3, 4].

The prevalence of this disease is about one percent in the world and usually occurs during middle age. It is more common in women [2, 5].

The rate of osteoporosis, infection, cancer, digestive diseases, cardiovascular diseases, and hypertension [1, 2] are higher in RA. Also using medications such as corticoesteroids that may increase the risk of osteoporosis is higher in these patients [2].

The term conjugated linoleic acids describes the mixture of positional and geometric isomers of linoleic acid, in which the most common and health-associated active isomers are c9, t11-CLA and t10, c12-CLA [6]. CLA is produced in the rumen as a result of incomplete biohydrogenation of linoleic acid. It is also produced during the commercial manufacture of dairy products [6].

Many studies have been carried out on the role of CLA in cancer, immune function, oxidative stress, atherosclerosis, and metabolism of fatty acids and lipids, the formation and composition of bones, obesity, and rheumatoid arthritis [7–11]. According to the theory of Park *et al*., CLA may be a substrate for cyclooxygenase that share the same continuum elongating and non-saturation systems with linoleic acid, linolenic acid, and arachidonic acid [12]. In a study on human osteoarthritis chondrocytes, it was shown that CLA significantly reduces PGE_2 and NO inflammatory mediators just like EPA [13]. CLA consumption reduces the amount of body fat by increasing protein, ash and body water [12, 14, 15]. The fact that in some studies the consumption of CLA has increased the amount of ash in the body may indicate the effects of CLA on the prevention or treatment of osteoporosis [15]. In a research on the effects of CLA and exercise on pelvic vertebrae and femoral diaphyseal, positive effects on bone formation parameters, such as ALK-P bone activity and PGE_2, were seen. In contrast, it had no effect on other indicators such as osteocalcin and insulin-like growth factor [16]. A study has shown that CLA modulates bone resorption by stimulating cytokines such as IL-6 and LTB4. It also showed that CLA probably affects bone metabolism through IL-6 and activation of NFκB receptors [17]. However, two clinical studies did not show a positive effect on bone mass [18, 19] while the rest of the studies showed positive correlation between CLA and bone mineral density [20, 21]. CLA improves calcium absorption. Although it did not have a significant effect on bone formation or bone resorption parameters in another report [22]. These observations were later approved on colon adenoma carcinoma cells of humans [23–25].

CLA in the balanced ratio with other dietary PUFA has increased the amount of bone formation in the femoral [26]. These studies showed that the amount of CLA effects depends on the content and the type of its isomers and the amount of fatty acids in the diet [27].

In all animal studies and studies concluded on cell culture media, it can be observed that this fatty acid modulates the immune system and reduces NO and PGE_2 and consequently the inflammation [28, 29]. In other studies conducted on mice, guinea pigs, and chickens, it was shown that CLA changes the fatty acid composition of bone and increases the concentration of n-3 fatty acids in bone and reduce the production of PGE_2 just like n-3 fatty acids. As a result, it can affect the formation or bone resorption [30, 31]. Various fatty acid isomers of CLA have different effects. But it seems that CLA fatty acids in the amount of 3 g of 11-*trans*,9-*cis* and 12-*trans*,10-*cis* isomers with a ratio of 50:50, especially in the form of glycerinated isomers as shown in our study, are more effective in the process of reducing inflammation and uptake of calcium from the bones and modulation of the immune system in humans [7, 27].

Several studies have been conducted on cell culture and animals which have shown the potential beneficial effects of CLA FA on bone markers and since there is no study on the effects of CLA on bone markers in autoimmune diseases in humans (especially in rheumatoid arthritis disease in which it is more common compared to other autoimmune diseases) the result of this study could be beneficial not only in reducing joint destruction in RA patients but it can also be effective in all autoimmune diseases, and bone disorders [29, 34]. Therefore, this research was done in order to help patients with RA as well as all patients with autoimmune and bone diseases.

Materials and Methods

A randomized double-blind placebo-controlled trial was conducted over a 12-week period in patients with active RA. The inclusion criteria were ages between 19–69 years, having RA for at least 2 years and fulfilling ACR criteria for RA [35, 36]. Data on dietary habits, dietary supplements, drug history and smoking habits were obtained by face-to-face interviews.

A written informed consent was obtained from all the participants before the study. The research protocol was approved by the Ethics Committee of Tehran University of Medical Sciences. 52 subjects were divided into 2 groups using method of random permutation. Two groups in terms of the type of supplement intake were as follows:

Group I received CLA, 2.5 g daily as two capsules, containing 2 g both *cis*-9,*trans*- 11 CLA and *trans*-10,*cis*-12 triglyceride type CLA in equal proportions.

Group II received standard treatment plus 2 placebo 1.25 g capsules containing sunflower oil with High oleic

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acid. The placebos were completely similar to CLA Capsules. CLA and its placebo were produced by the Lipid Nutrition Company.

The filling out of general information questionnaires and measuring of height and weight were carried out before and after the intervention. In order to investigate variations in their food intake and to control diet-related confounding factors, three 24-h dietary record questionnaires for three consecutive days (two ordinary days and a holiday) were taken before and after the study. They were analyzed by Food Processor (version 4, USA).

The subjects were asked not to alter their usual diet and physical activities throughout the study. Neither the researchers nor the patients were aware whether the patients belong to CLA or placebo group.

Body weight was measured in the fasting state with light clothing and without shoes using Seca scale (Seca, Hamburg, Germany). Also height was measured without shoes using a stadiometer attached to the scale. BMI was calculated by dividing the weight in kilogram by the square of height in meter. Physical activity was evaluated by International Physical Activity Questionnaire (IPAQ).

Patients were under treatment with disease modifying anti-rheumatic drugs (DMARD: methotrexate, hydroxychloroquine and prednisolone <10 mg/day) and not receiving anti-inflammatory drugs (NSAIDs) as far as possible. Changing the amount of NSAIDs during the study period was allowed, but it had to be reported and recorded.

At the beginning and after 12 weeks of the study, 10 ml fasting venous blood was taken from all patients. The Laboratory tests measurement including telopeptides C and osteocalcin were carried out in laboratories of Biochemistry and Nutrition in the faculty of Nutrition and dietetics of Tehran's University of Medical Sciences. Patients with active RA were treated for 3 months and they were controlled by phone weekly. At the end of every month the patients were controlled. As well Measuring Biochemical and immunological blood indices included: measurement of telopeptides C, osteocalcin, MMP3 by the ELISA method and using a kit made by Bioassay Technology Laboratory Company, Measurement of PGE_2 by a competitive enzymatic immunoassay method and using a monoclonal kit made by the Cayman Company (England), measurement of IGF-1 levels by immunohistochemical method based on sandwich method and using a kit made by Immunotech Beckman Coulter (France), measurement of ALK-P of bone using two simultaneous methods: (1) heating for inactivation of ALK-P, (2) heating and inhibition by urea that the difference between the two numbers shows the amount of ALK-P in bone.

Statistical Analysis

Statistical analysis was carried out using SPSS software version 18 (SPSS, Chicago, IL, USA). Kolmogorov–Smirnov test was used to determine data compliance with the normal distribution. Quantitative variables were compared between the two groups at baseline and at the end of the study using an independent *t* test. Quantitative variables before and after treatment within each group were compared with the paired *t* test. Qualitative variables were analyzed with Chi-squared tests. All values were reported based on the mean ± SE. A *P* value of <0.05 was considered as the statistical significance level.

Results

Of the 52 patients with active RA participating in this study, 5 patients were excluded due to the change in medication (2 in the case group and 3 in the control group) and lack of intake of regular doses of the supplements (2 in case and 1 in control). Of 44 patients studied with active rheumatoid arthritis 38 were women (86%) and 6 were men (14%). The mean age of participants was 46.48 ± 12.53 years and its domain was 21–69 years. The mean duration of RA in patients participating in the study was 9.43 ± 7.64 and its domain was 2–42 years. None of the patients were smokers and they did not use drugs (opium).

Characteristics of active RA patients participating in the study are provided in Table 1. There were no significant differences between the groups in terms of distribution of age and duration of rheumatoid arthritis using the *t* test. Besides, there was no significant difference between the groups in terms of the distribution of gender using the Chi-square test.

Table 1 Findings related to general information and the diet of patients with active rheumatoid arthritis before the intervention

Variables	Groups		*P* value
	CLAs ($n = 22$)	Placebo ($n = 22$)	
Number (person)	22	22	NS*
Female	19 (%86)	19 (%86)	NS$^{\Psi}$
Male	3	3	
Age (years)	46.2 ± 13.1	47.9 ± 11.1	NS*
Duration of RA (years)	9.9 ± 8.4	8.9 ± 9.6	NS*

Data are presented as means ± SE

P value <0.05 is significant

* *P* independent *t* test

$^{\Psi}$ *P* Chi-squared test

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Table 2 Means and standard deviations of the body mass index (BMI) of studied patients with active rheumatoid arthritis before and after months of intervention (mean ± SD)

Variables	Groups		*P* value
	Placebo ($n = 22$)	CLAs ($n = 22$)	
BMI (kg/m^2)			
Before of intervention	28.4 ± 3.9	27.2 ± 4.6	NS$^{\psi}$
After of intervention	28.4 ± 3.9	27.2 ± 4.6	
P value*	NS*	NS	

Data are presented as means ± SE

P value <0.05 is significant

* *P* > 0.05 independent *t* test

* *P* within group comparison (paired *t* test)

$^{\psi}$ *P* between groups comparison (independent *t* test)

Before the intervention, the mean of BMI in these patients was 27.8 ± 4.2 kg/m^2 and its domain was 19.6 ± 38.3 kg/m^2. Mean and standard deviations of patients' BMI, before and after intervention, have been provided in Table 2 for both studied groups. As it can be observed, there was no significant difference between the means of the body mass index of participants in the studied group at the beginning of the study. Also after 3 months of supplementation, no significant difference was observed between the two groups in terms of BMI distribution using a BMI independent *t* test.

Measurement of all nutrition assessment parameters such as energy, protein, carbohydrate, received fiber, different kinds of fatty acids, anti-oxidants (vitamins A, C and E, selenium), and mineral elements (zinc, calcium, sodium, potassium, magnesium, etc.), using an independent *t* test from the diet, showed that there are no significant differences between these parameters at the beginning of the study. In addition, the independent *t* test showed that there were no significant differences in the means of the above parameters in the placebo and CLA groups (data have not been shown).

Table 3 shows means and standard deviations of the activity of bone alkaline phosphatase enzyme, PGE_2, MMP3, IGF1, osteocalcin and C-telopeptidase.

Changes of bone ALK-P within the group with 3 months of supplementation was statistically significant between groups compared to pre-intervention ($P > 0.05$) and respectively bone ALK-P decreased with in CLA and placebo groups ($P = 0.03$ and $P = 0.001$).

Changes of PGE_2 within the group with 3 months of supplementation were not statistically significant within the two groups compared to pre-intervention. Changes between CLA and placebo groups were not significant (Table 4).

After 3 months of intervention the differences between groups for MMP3 using ANCOVA were not statistically significant. Changes of MMP3 within each group after 3 months of supplementation were not statistically significant.

Table 3 Means and standard deviations of the activity of bone alkaline phosphatase enzyme, PGE2, MMP3 and IGF 1, Osteocalcin, C-telopeptide of type 1 collagen in patients with active rheumatoid arthritis before and after 3 months of supplementation (mean ± SD)

Variables	Groups		*P* value
	Placebo ($n = 22$)	CLAs ($n = 22$)	
ALK-P (U/l)			
Before intervention	22.23 ± 3.72	22.11 ± 3.17	
After intervention	18.99 ± 4.10	20.13 ± 3.13	
	$P = 0.001$*	$P = 0.03$*	NS†
PGE2 (pg/ml)			
Before intervention	607.91 ± 106.77	623.28 ± 102.59	NS
After intervention	607.82 ± 84.79	613.45 ± 92.06	
	NS	$P = 0.5$	
MMP3 (ng/ml)			
Before intervention	65.48 ± 38.96	72.93 ± 40.41	
After intervention	87.40 ± 53.53	69.55 ± 48.29	
	$p = 0.059$	$p = 0.5$	$p = 0.1$
IGF1 (ng/ml)	250.95 ± 122.1	194.9 ± 76.3	$p = 0.016$
Before intervention	163.73 ± 92.37	209.5 ± 119.70	
After intervention	($p = 0.009$)	($p = 0.1$)	
Osteocalcin (ng/ml)			
Before intervention	33.53 ± 24.1	34.14 ± 25.5	$P = 0.018$
After intervention	29.68 ± 23.1	36.18 ± 27.3	
	$p = 0.065$	$p = 0.1$	
C-telopeptide of type- 1 collagen (ng/ml)			
Before intervention	42.31 ± 23.3	50.43 ± 40.7	$P = 0.045$
After intervention	44.9 ± 24.7	47.94 ± 39.5	
	NS	$p = 0.01$	

Data are presented as means ± SE

P value <0.05 is significant

* *P* within group comparison (paired *t* test)

† *P* between the groups comparison (independent *t* test of mean differences)

At the end of the study, IGF1 significantly decreased in the placebo group ($P < 0.009$) but it had an insignificant increase in CLA group; in a way that after three months of intervention, the difference between CLA and placebo groups was statistically significant ($P = 0.01$).

After 3 months of supplementation, osteocalcin decreased in the placebo group but this reduction was not significant ($P < 0.06$), but in the CLA group, the increase

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Table 4 Inclusion and exclusion criteria of the study on the effect of conjugated linoleic acid (CLA) on some bone markers in patients with active rheumatoid arthritis

Inclusion criteria	Exclusion criteria
Being 19 to 69 years old	Unwillingness to cooperate
Individuals with rheumatoid arthritis according to ACR criteria	CLA or placebo supplement discontinuation (compliance less than 80%)
Active rheumatoid arthritis based on clinical diagnosis	Changes in diet or physical activity, for any reason
Disease diagnosis being in at least 2 years ago	Changes in type and amount of daily medication
Not having underlying disease such as diabetes and hyperlipidemia	Kidney, liver, thyroid and parathyroid disease
Using a fixed amount of anti-inflammatory drugs in last two months	
Having consent to collaborate	
Lack of pregnancy and lactation	
Lack of smoking and alcohol consumption	
Lack of Multivitamin supplements and antioxidants consumption in the last three months	
Lack of antihypertensive drug consumption	
Lack of oral contraceptives consumption	

was insignificant; in a way that after three months of intervention, the difference between CLA and placebo groups was statistically significant ($P = 0.01$).

Changes in C-telopeptides within the placebo group after 3 months of supplementation was not statistically significant compared to pre-intervention and this reduction in CLA was statistically significant ($P = 0.01$). Moreover, after three months of intervention the difference between the placebo group with the CLA group was statistically significant ($P = 0.04$). In terms of supplementation, no statistically significant difference was observed between the groups in terms of enzyme activity of ALK-P bone, PGE_2, and MMP3.

Discussion

In the present study, which aimed at evaluating the effects of CLA on bone markers in patients with rheumatoid arthritis, there was no significant difference in nutritional intake and physical activity between the two groups of placebo and CLA, before and after the intervention, in terms of a variety of nutrients including energy, protein, carbohydrate, fat, various minerals, and vitamins. Also there were no significant differences between the two groups in other factors such as sex, age, BMI, and disease duration.

In the present study, there were no statistically significant differences between the groups for ALK-P bone enzyme activity, PGE_2 and MMP3; however the differences between the two groups for osteocalcin, IGF1 and telopeptides C were significant. In two groups changes in bone ALK-P within the each group at 3 months of supplementation was statistically significant compared to pre-intervention by the paired t test ($P < 0.01$) and respectively decreased in CLA and placebo groups ($P = 0.03$ and $P = 0.001$).

Serum amounts of bone alkaline phosphatase, which is often used as a marker of bone formation, increased in RA patients [36]. In other studies carried out on mice, guinea pigs, and chickens, it was shown that CLA changes the fatty acids composition of bones and increases the concentration of n-3 FA and reduces the production of PGE_2 just like n-3 FA in bones. As a result, it may affect the formation or resorption of bones [30, 34, 37]. CLA consumption reduces the amount of body fat by increasing protein, ash, and body's water [7, 38–40]. The fact that the consumption of CLA in some studies increases the amount of ash in the body may indicate the effects of CLA on the prevention or treatment of osteoporosis [41, 42].

In various studies, CLA positive effects on bone formation parameters such as ALK-P bone activity and PGE_2 have been observed [30, 32, 38–43]. In contrast, CLA had no effect or a negative effect on other indicators such as osteocalcin and insulin-like growth factor [22]. In the present study, although there was no significant effect on ALK-P and PGE_2, but IGF1 showed a significant increase in the CLA group compared to the placebo group and osteocalcin, which is considered as a bone formation marker and helps the process of mineralization had an insignificant reduction in the placebo group. IGF1 had an insignificant increase in the CLA group and generally, the changes were significant between Placebo and CLA groups which indicates beneficial effect of CLA in mineralization process. Telopeptides c is considered as a marker of bone resorption [36] and in this study had a significant reduction in the CLA group. Also its change was significant between Placebo and CLA groups, which indicates CLA intake in RA patients can help prevent bone loss by lowering the C-telopeptides and slow down the destruction process. It should be noted that the effects of CLA on bone resorption are contradictory [44]. So we suggest more studies with

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larger samples size and longer time, in association with bone mineral density measurement. Studies have shown that CLA modulates bone resorption stimulating cytokines such as IL-6 and LTB4 and probably affects bone metabolism through IL-6 and activated pathways of NFκB receptor [45, 46]. However, two clinical studies did not show a positive effect on bone mass [19, 47], while the rest showed a positive correlation between bone mineral density and CLA [48, 49]. Kelly and colleagues [33] showed that CLA improves calcium absorption, although they did not have significant effect on bone formation and bone resorption parameters in the same report [33]. These observations were later approved on Colon adenoma carcinoma cells of humans (Caco-2) [23–25].

Watkins *et al*. [50] reported that adding CLA in balanced amounts of dietary PUFA can enhance the amount of bone formation in the femur. These researches showed that CLA impacts depend on its isomers, type of fat and content of fatty acids in the diet [50]. There are some reports that the isomer t10, c12-CLA reduces the fat storage and its synthesis in adipocytes [51]. Kim *et al*. [73] have showed *trans*-10,*cis*-12 CLA promotes bone formation by inhibiting adipogenesis by peroxisome proliferator activated receptor-γ (PPARγ) dependent mechanisms and by directly enhancing osteoblastogenesis from bone marrow mesenchymal stem cells [52]. These mechanisms make CLA a good candidate for controlling bone fat, because fat storage in bone marrow is dependent on the reduction of bone formation. CLA also increases the expression of leptin and its circulation [53–56]. Leptin has a positive effect on the bone formation by affecting the central nervous system, stimulation of bone marrow stromal cells and inhibition of osteoclast cells [57, 58]. In addition CLA reduces the loss of bone with aging, by decreasing inflammatory markers and Osteoclast cells expression [16, 59].

CLA is anti-proliferative [60] but their exact mechanism of action is not specified. According to the theory of Park and his colleagues, CLA may be a substrate for cyclooxygenase that share same unsaturated chain elongation systems with LA and AA. CLA competes with other fatty acids in eicosanoids production. It has shown that using a diet containing 1% CLA in mice with colon cancer (induced by dimethyl hydrazine) reduces the level of PGE_2 and thromboxane B2 in colonic mucosal and decrease inflammation [11]. In the present study, we observed a decrease in PGE_2 amount in the group receiving CLA but this reduction was not statistically significant. It may be due to the need for more participants or longer study duration.

CLA have inhibited the synthesis of eicosanoids derived from Arachidonic Acid (AA) in mouse bone in culture media [22, 61]. On the other hand, it was effective on cyclooxygenase enzyme system especially on Cyclooxygenase-2 which is responsible for the conversion of AA to inflammatory prostaglandins [62–64]. CLA can inhibit the activity or expression of Cyclooxygenase 2 in bone and reduce bone resorption. The production of parathyroid hormone, insulin-like growth factor type 1, prostaglandin related to bone resorption may be affected by CLA. Also it can affect on the bone cells by the altering the performance of PGE_2 receptors [65–68].

Deguire and colleagues (2012) observed that the status of 9-*cis*,11-*trans* CLA isomer of reflecting red blood cells in the group was receiving CLA diet in long-term (4 months) was positively associated with BMD [69].

Rhonda and colleague [70] examined the relationship between receiving a CLA diet and BMD in postmenopausal women and they observed that there is a positive association between CLA dietary consumption and BMD [70]. Yooheon Park *et al*. (2013) studied CLA and calcium co-supplementation effects on bone health in ovariectomized mice. The result of their study showed that CLA significantly increased bone markers without major changes in bone mineral composition in the femur compared to respective controls. CLA treatment increased serum parathyroid hormone (PTH) significantly, while serum 1, 25-dihydroxyvitamin D3 concentration and ALK-P (as our study) were not changed by CLA. Meanwhile, CLA significantly reduced femur tartrate resistant acid phosphatase (TRAP) activity, suggesting potential reduction of osteoclastogenesis [71].

In Rahman *et al*. [72] study on the effect of CLA on the ovariectomy-induced bone loss in mice, CLA caused modulating both osteoclastogenesis and osteoblastogenesis by inhibiting of pro-osteoclastogenic RANKL and stimulating decoy receptor of RANKL, OPG expression. CLA also inhibited proinflammatory cytokine and enhanced anti-inflammatory cytokine production of LPS-stimulated splenocytes and bone marrow cells. Moreover, CLA suppressed osteoclast differentiation in Bone Mass (BM) and stimulated osteoblast differentiation in BM stromal cells as confirmed by TRAP and Alizarin Red staining, respectively [72].

However, in the study of Doyle and his colleagues [18], by giving CLA and a placebo supplement, no significant effect was observed on bone formation markers, including osteocalcin and serum alkaline phosphatase and serum markers of bone resorption including c-telopeptides serum and urinary telopeptides and urinary and serum calcium levels after 8 weeks[18]. Their results are against our study that may due to shorter duration of the study and differences in the samples which was carried out on postmenopausal women.

Kim *et al*. [73] showed *trans*-10,*cis*-12 CLA promotes osteoblastogenesis via SMAD mediated mechanism in bone marrow mesenchymal stem cells. Results of their studies showed that SMAD8 plays a role in CLA's effect

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on osteoblastogenesis. Also, due to the inverse correlation of differentiation between adipocytes and osteoblasts in mesenchymal stem cells, inhibiting SMAD8 may influence adipogenesis in mesenchymal stem cells. CLA may increase osteoblastogenesis via another different mechanisms, such as other SMAD or Wnt10b. Since BMP signals through heterodimers of SMAD, other SMAD (SMAD 1, 4, 5 and 8) may also have significantly influence, as does SMAD8. Meanwhile, Wnt signal involves complex interactions among various proteins and activation of Wnt1, Wnt2, and Wnt3a induced alkaline phosphatase [73].

Metalloproteinases lead to destruction of the cartilage proteoglycan and other matrix molecules. Imbalance between metalloproteinases and their inhibitors may lead to destruction of joint tissues. Concentration of metalloproteinases in the synovial fluid of patients with RA increases and especially MMP3 increases in patients with active RA and it has used as an indicator of response to treatment [73]. Also in this study an insignificant increase in MMP3 in the placebo group and an insignificant reduction in the CLA group were observed. Difference observed between groups in terms of MMP3 was not significant [74].

Conclusion

According to the results of this study, there is the potential of beneficial effects of CLA on bone markers in patients with rheumatoid arthritis which is more common compared to other autoimmune diseases. So it may be useful in the prevention and reduction of osteoporosis in RA patients. Therefore, in order to help patients with RA as well as all patients with autoimmune and bone diseases, further studies of CLA isomers in different amounts and larger sample size associated with BMD in human is recommended.

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Compliance with Ethical Standards

Conflict of interest None of the authors report conflicting interests.

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Ethical approval The study was approved by the respective research ethics committees and medicinal regulatory agencies in each country (No IRCT201207109472N4). Informed written consent was obtained from the patient before recruitment.

Transparency DP affirms that the manuscript is an honest, accurate, and transparent account of the study; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing No additional data available.

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