Original Article

Effect of Dietary Supplementation with Conjugated Linoleic Acid on Bone Mineral Density, Bone Metabolism Markers and Inflammatory Markers in Healthy Post-menopausal Women: a Randomized Double Blind Placebo Controlled Trial

Reza Tavakoli Darestani [1], Azadeh Tavakoli Darestani [2], Gholam Hossein Kazemian [*1], Farideh Tahbaz [3], Farideh Najafi [1]

[1] Department of Orthopedic Surgery, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Nutritionist, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Human Nutrition, National Nutrition and Food Technology Research Institute, Tehran, Iran

Abstract

Introduction: Conjugated linoleic acid (CLA) has been shown to positively influence calcium and bone metabolism in experimental animals and cell culture, but there are limited human data available.
Material and Methods: The study consisted of a double-blind, placebo-controlled trial in which 76 healthy post-menopausal women (aged 55.1) were randomly assigned to receive daily either four capsules CLA G80 containing 3.2 g isomer blend (50:50% cis-9, trans-11: trans-10, cis-12 isomers) or four capsules containing high oleic sunflower oil as placebo for 12 weeks. Urine and blood samples were collected at weeks 0 and 12 and were analyzed for biomarkers of calcium and bone metabolism and inflammatory markers (TNF-α and IL-6). Subjects completed 3-days dietary records during the trial, in weeks 0 (baseline), 6 and 12.
Results: Supplementation with 3.2 g CLA isomer blend (50:50% cis-9,trans-11:trans-10,cis-12 isomers) for 12 weeks had no significant effects on markers of bone formation (serum osteocalcin, bone-specific alkaline phosphatase) or bone resorption (urine C-telopeptide-related fraction of type 1 collagen degradation products), PTH, urinary calcium, urinary creatinine and CTP to creatinine ratio. And serum interleukine-6 did not change significantly over 12 weeks in postmenopausal women.
Conclusion: Under the conditions tested in this double-blind, placebo-controlled trial in postmenopausal women, 3.2 g CLA isomer blend (50:50% cis-9, trans-11: trans-10, cis-12 isomers) did not affect markers of bone metabolism and calcium.
Key Words: Conjugated linoleic acid (CLA), post-menopausal women, bone mineral density, IL-6

***Corresponding Author:** Gholam Hossein Kazemian, Department of Orthopedic Surgery, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: gh_kazemian@yahoo.com, Tel: +98 9121168246.

Please cite this article as: Tavakoli Darestani R, Tavakoli Darestani A, Kazemian GH, Tahbaz F, Najafi F. Effect of Dietary Supplementation with Conjugated Linoleic Acid on Bone Mineral Density, Bone Metabolism Markers and Inflammatory Markers in Healthy Post-menopausal Women: a Randomized Double Blind Placebo Controlled Trial. Novel Biomed 2013;1(3):66-72.

Introduction

Women normally spend one-third of their lives in postmenopausal phase[1]. Especially in the first decade after menopause, bone density loss is observed[2]. Shortage of ovarian hormones, particularly estrogen efficiency, is the most common cause in bone density loss after menopause. This factor exposes postmenopausal women to higher risk of osteoporosis[3]. In the elders, especially in women, it has been seen that osteoporosis due to bone density loss and high bone fragility lead to osteoporotic fractures[4].

Today, hormone therapy and inhibitory medicines of bone re-absorption are used in order to prevent and treat osteoporosis[5]. But, due to possible consequences of estrogen therapy such as breast cancer and endometrial adenocarcinoma, the adoption of this kind of treatment in postmenopausal is very low and only 3% to 8% of the women accept this kind of treatment[6]. Prevention of osteoporosis is easier than its treatment. Nutrition and lifestyle management are used in order to minimize bone density loss, decrease the need for medication and prevent osteoporosis. The effect of nutritional interventions on bone density regulation is well known[7].

Over the past few years, there has been considerably much attention to biological benefits of conjugated linoleic acid (CLA) on health care[8], for example anti-obesity function in animals[9-11] and humans[12-17], anti-cancer effects, anti-tumor effects[18-21], the risk reduction of atherosclerosis[22-25], reduction of the risk factors of diabetes and high blood pressure[26-27], energy metabolism, positive effects on immune performance[28] and anti-inflammatory properties[29-32]. New properties of conjugated linoleic acid (CLA) are the increase of immune functions and positive properties in formation of bone in animal models.

Useful properties of conjugated linoleic acid (CLA) are not provided by diet lonely and there is need for food supplements. There are 28 different isomers in conjugated linoleic acid (CLA) which main form of this acid is found in food, cis-9 and trans-11 as it is called Rummenic Acid. This isomer is 90% of conjugated linoleic acid in food[33]. Health benefits of conjugated linoleic acid isomers are mainly related to two kinds of isomers cis-9, trans-11 and cis-12.

According to previous studies, there is an incoherence which isomers of conjugated linoleic acid (CLA) is more effective on bone metabolic bone[34]. Recent surveys on animals show that conjugated linoleic acid (CLA) can minimize bone density loss by decreasing the level of prostaglandin E2 in the bone issue[35-36]. Prostaglandin E2 is synthesized from arachidonic acid by cyclophosphamide oxygenase enzyme and effects on bone metabolism. The restrain or stimulation in bone formation and re-absorption is dependent on the prostaglandin E2 concentration. It leads to increased insulin- like growth factor (IGF) at low levels and regulates protein expression to insulin- like growth factor (IGFBP) and thus stimulates bond formations[37,38]. In high doses, it causes restraining the synthesis of collagen, decreasing mRNA in the bone, restraining osteoblast function and finally decreases the formation and density of bones[37].

On one hand, increasing the age and on the other hand, decreasing estrogen in postmenopausal women along with increase in producing prostaglandin E2 and inflammatory cytokines such as Tumor necrosis factor alpha, interleukin-6 and beta 1 by immune cells[39-42] results to the increase the activity of osteoclast and bone re- absorption in postmenopausal[43]. These cytokines lead to increase cyclo-oxygenase expression 2 in osteoblastic and the production of prostaglandin E2[44,45]. On the other hand, conjugated linoleic acid (CLA) causes to increase the absorption of the calcium[46,47]. In previous studies, there are shown the decreasing effects of inflammatory cytokines activities by conjugated linoleic acid (CLA) in animal models[48].

Therefore, due to mentioned subject, the evidences about the effect of linoleic acid (CLA) on bone density and its metabolism in animal and human model are mysterious. On the other hand, there is not performed any survey based on this research in order to evaluate those effects on postmenopausal women. This survey is conducted to determine the supplementary effect of conjugated linoleic acid (CLA) with similar mixture from known isomers trans- 10, cis- 12, cis- 9, cis- 11 on the bone mineral density, its metabolism and inflammatory indexes in healthy postmenopausal women.

Materials and Methods

In this clinical trial 76 women in the age of 45-65 with at least one and maximum 10 years spent their menopause period and their body mass index were 20-32 kg/m2, were studied. Exclusion criteria included sever osteoporosis (t score below -2.5 for hip and spine), bone disease, smoking, using alcohol and drugs, risk of fracture in recent three months, heart disease/ cardiovascular, hepatic disease, gastrointestinal, diabetes, hypothyroidism or hyperthyroidism, hormone therapy, dietary supplements for weight loss, omega 3 supplements, medicines increasing bone, bone re-absorption inhibitors, corticosteroids, anticonvulsant medicines and lipid and blood sugar lowering drugs.

The written informed consents were obtained from all patients. Patients were randomly divided into two groups. First group received CLA supplement, daily 4 capsules (each capsule 1 gram, totally 3.2 gram from the mixture 2 isomers cis- 9, trans- 11and trans- 9 and cis- 12 conjugated linoleic acid) and second group received some placebo, daily 4 capsules (each capsule was one gram containing sunflower oil with oleic acid). In this survey, the patients received the supplements and placebos for twelve weeks. CLA supplements used in this study were as follows: Clarinol G80 coated with a transparent gel coat containing 80% CLA (Dutch firm Lipid Nutrition Loders Croklaan B. V.). The patients were trained to take 3 capsules with each meal and 1 capsule before going to bed.

The amounts of supplement acceptance by the patients were followed up by weekly calls and capsules remaining in packages were investigated at each visit, also patients were questioned about their problems. In these contacts, some issues and probable problems were addressed such as intolerance to supplements and other problems, probable change in use of foods, new disease and changes in physical activity. If any condition would happen, the patient would be omitted from the survey or his results would not be used for final analysis. At the end of the sixth week and twelfth week, the capsules were evaluated in terms of patients' compliance, by counting remaining capsules.

Blood Biochemical Assessment

Serum concentrations of inflammatory markers, tumor necrosis factor alpha, interleukin 6, metabolic markers of the bone including bone alkaline phosphate, osteocalcin, C- urinary telopeptide (CTP) and parathyroid hormone (PHT), urinary calcium and urinary creatinine were measured.

The levels of the serum IL-6 and TNF-α were investigated by ELISA which is the research kit (ELISA kit for human IL-6 and ELISA kit for human TNF-α, French Diaclon Firm). The measurement method sensitivity was lower than 2 pg.ml and 8 pg.ml, respectively. The accuracy of in-test in mentioned measurement was 5.3 and 6.1 respectively based on the percentage of in-test changes. C- telopeptide level in urine samples was determined by EIA and the bone-specific alkaline phosphate levels in serum were determined by using of immune enzymatic by research kits of Inc. IDS Bolton, UK. The sensitivity of measured method was 50 and 0.7 micrograms per liter. In-test accuracy in above measurement was 4.1 and 4.9, respectively based on the variation coefficient.

Serum osteocalcin levels were determined by using ELISA by research kits of IDS Inc. Bolton, England). The sensitivity of mention method was 0.5 ng.ml. The accuracy of in-test in mentioned measurement was 1.7 based on the variation coefficient.

The levels of serum parathyroid hormone (PTH) were measured by research kits of IDS Inc, Bolton, England by using of ELISA method. The sensitivity of mention method was 0.5pm.l. The accuracy of in-test in mentioned measurement was 5.5 based on the variation coefficient.

Urinary creatinine level was measured by chemical colorimetric method (Pars Test Inc., Tehran, Iran). The sensitivity of mention method was 6 mg.dl. The accuracy of in-test in mentioned measurement was 1.2 based on the variation coefficient. Urinary calcium was measured by chemical colorimetric method (Pars Test Inc., Tehran, Iran). The sensitivity of mention method was 0.2 mg.dl. The accuracy of in-test in mentioned measurement was 2.3 based on the variation coefficient.

Statistical Methods

To compare results, there were used t-test, Wilcoxson, Mann-Whitney U, Wilcoxson and ANOVA. The

meaningful level was considered as 0.05. There was used statistical software SPSS, version 16 to analysis data.

Results

First of all, 76 healthy postmenopausal women participated in this survey. There were 38 women in the placebo group and 38 women in the group of supplement receivers. The number of 4 women from the first group (2 persons because of headache, 2 persons because of digestive problems or bellyache) and 5 women from second group (4 persons because of digestive problems and 1 person for traveling) were excluded from the survey. Therefore, total women participated in this survey were 67 persons. The amounts of capsules based on their counting were determined for first group (receiving CLA supplements) 85% and for second group (placebo group) 75%.

Patients in two groups were similar in terms of age, time duration of menopause, height, body mass index, waist circumference, physical activity, systolic and diastolic blood pressure. In this survey, the changes related to bone mineral density and bone mineral content were not significant in two groups (Table 1). Tumor necrosis factor- alpha levels decreased after 12 week consuming CLA supplements (1.97 pg.ml). But the rate of changes of IL-6 was not statistically significant (Table 2, Figure 1).

Table 1: Bone mineral density in post-menopausal women initially and after 12 weeks.

Body Composition	Placebo(n=33)		CLA supplement (n=34)		
	Baseline	After 12 weeks	Baseline	After 12 weeks	P value
Bone mineral content(gr)	1997/3±264	1967/2±251	1942/85±294	1936±278	0.76
Bone mineral density (gr/cm^2)	1/1±0/09	1/1±0/09	1/08±0/09	1/09±0/09	0.2

Changes in serum osteocalcin, serum bone alkaline phosphatase, PTH, C-telopeptide, urinary calcium, urinary calcium to creatinine ration were not statistically significant after 12 week consuming CLA supplements (Table 3).

Table 2: Level of inflammatory markers in post-menopausal women initially and after 12 weeks.

Inflammatory markers	Placebo (n=33)		CLA supplement (n=34)		
	Baseline	After 12 weeks	Baseline	After 12 weeks	P value
TNF-a (pg/ml)	7/8±3/9	8/7±3/4	6/8±3/7	4/7±2/2	0.00
IL-6 (pg/ml)	0/95±0/8	1/12±0/7	1/11±0/7	1/03±0/6	0.07

Table 3: Serum and urinary bone metabolism marker levels in post-menopausal women initially and after 12 weeks.

Bone metabolism markers	Placebo(n=33)		CLA supplement (n=34)		
	Baseline	After 12 weeks	Baseline	After 12 weeks	P value
Osteocalcin (ng/ml)	16±7/9	18/75±8/3	15± 7/2	15/7±6/5	0.16
Bone alkaline phosphatase (µg/l)	19/5±8	18/6±6/1	18/7±6	18/2±7/5	0.75
PTH (pmol/l)	2/3±1/9	2/9±1/2	2/7±1/6	3/2±1/8	0.93
Urinary creatinine (mmol/l)	12/3±5/1	12/9±5/1	12/2±6	14/4±3/3	0.14
Urinary calcium (mmol/l)	4/2±1/2	3/7±0/9	3/8±1/07	3/4±0/84	0.41
Urinary calcium to creatinine ratio	0/39±0/03	0/35±0/04	0/35±0/02	0/25±0/01	0.23
Urinary CTP (µg/l)	2903/5±1819	3402/4±2041	2702±1353	3339±1639	0.75
CTP/Cr (µg/mmol)	236/1±106	266±208/9	250/5±128/9	234/1±116/7	0.35

Discussion

In this survey, CLA supplement did not have any effect on urinary calcium in postmenopausal women participated in the survey. The only survey on the effect of CLA supplement on serum calcium and

urinary calcium is related to Doyle et al in 2005 and its results are similar to ours. In Kelly et al study, the calcium intestinal absorption in young mice were significantly increased by using of CAL supplements and food diet rich in fatty acid PUFA, n-3. But, this effect was not observed in food diet rich in fatty acid PUFA, n-6. CLA effects on bones are dependent on the amount of PUFA fatty acids in food diets. Both fatty acids n-3 and n-6 affect on cytokines, prostaglandins and, as a result, on the metabolism of bones. CLA is a competitive inhibitor with fatty acid PUFA, n-6. It prevents elongation and reduces the availability of substrate for the enzyme cyclo-oxygenase and causes decreasing prostaglandin E2 production. In the present study, the amount of intake the fatty acid PUFA n-6 was higher than fatty acid PUFA n-3. Lack of effect of CLA on bone metabolism markers and urinary calcium, despite of the decline in serum tumor necrosis factor-alpha, may be due to the inhibitory effect of high levels of fatty acid PUFA, N-6.

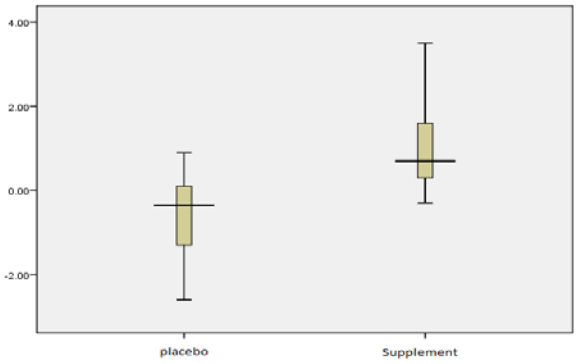

Figure 1: Changes of TNF-a level in each group of our study.

It is necessary to pay attention to special effects of CLA isomers as the lack of CLA effect on the bones may be related to the isomer kinds or used different percentages of two usual isomers of CLA (trans- 12, cis-9, cis-11). In the study performed by Park et al, the isomer with trans-10 and cis-12 of CLA leads to increased body ash in mice whereas the isomer cis-9 with trans-11 had no effect (10). Totally, we can conclude the isomer with trans-10 and cis-12 has suitable effect on bone metabolism; although, predominant isomer in food diet is cis-9 with trans-11 that 90% isomers in food resources (dairy and meat) is this kind of isomer.

Due to available direct and indirect evidences about probable effects of CLA on bone metabolism, we can conclude as follows:

1- Decreasing preinflammatory cytokines such as TNF-a, IL-1and IL-6 and increasing prostaglandin E2 and osteoclastogenesis
2- Regulating leptin and decreasing re-absorption and increasing bone formation
3- Regulating serum insulin level
4- Decreasing serum parathyroid hormone level and reducing the osteoclast activity

Osteoporosis or progressive loss of bone mass is happened due to the increase of bone osteoclastic activities comparing to its osteoplastic activities. However, the aging process is associated with increased pre-inflammatory cytokines such as TNF-a, IL-1, IL-6 and IL- Beta 1. These cytokines are known as main regulators of osteoclastic activities and bone re-absorption increase. These cytokines lead to increase the expression of cyclo-oxygenase 2 in stroma and osteoplastic cells and produce prostaglandin E2 as an essential factor in osteoclastogenesis[49]. In recent study, after 12 weeks using of supplementation with CLA, the level of TNF-alpha increased significantly but the changes of IL-6 was not statistically significant. In some surveys, CLA leads to decrease TNF-α levels[8,48,49]. On the other hand, some reports showed some increase or any change in TNF-α after using CLA[49]. Such a disharmony in reported results is related to the differences between isomers kinds, their purity, using different ratios of two isomers, duration of study and empirical models.

Another hypothesis about CLA is that prostaglandin E2 has involved in secretion of PTH and bone metabolism. One of the undesirable effects of estrogen deficiency, after menopause, is the negative balance of calcium which leads to form secondary hyper-parathyroidism. This condition causes to increase bone re-absorption. In conducted study on mice with polycystic kidney, there was observed the feeding with CLA for 8 weeks, compared to control group, in all mice induced the reduction of 60% in PTH and the secretion reduction of prostaglandin E2[50]. If CLA leads to decrease the biosynthesis of prostaglandin E2 in parathyroid tissue, there will be a risk to reduce the secretion of PTH. However, to prove this hypothesis, there is need to

perform some survey in animals and humans. In recent study, CLA supplement, compared to the placebo, has no effect on the secretion of PTH in postmenopausal women.

One of the limitations in present study is the measurement of conjugated linoleic acid levels in participants' serums showing the acceptance rates of patients correctly. The numbers of capsules used in this study were high because of low purity of CLA (about 80%). It was suggested, by using of supplements with higher purity, the used capsules are reduced in order to increase acceptance rates of persons and reduce their digestive problems. Due to the counting of capsules, the acceptance rates of capsules was 75% in placebo group and 85% in the group received supplement.

One of the main reasons in the various obtained results from CLA effects on bone and body composition in human and animal studies is using of CLA supplement dose. In human studies, used dose is daily between3-6 g; while in animal studies, used dose is 30 times higher than human studies[51]. If we are used the supplements with high purity, we can use upper dose from this supplement. Another reason for the lack of CLA effects on bone formation markers (such as osteocalcin, bone- specific alkaline phosphate) is short duration of this research (12 weeks). The time of re-monitoring of bone formation markers is 6 months after food and medicine interventions and it is suggested to consider bone re-absorption markers about 3-6 months after food and medicine intervention.

One of the advantages of this study is to investigate postmenopausal women as an aim group because there are little studies about this group. It is suggested to investigate bone turnover markers in postmenopausal women in the first few years after menopause, when bone turnover rate is high. As a result, this study suggests that consuming 3.2 g CLA supplement containing equivalent isomers such as cis-9, trans-10, trans-11 and cis-12 with ClarinolTM is safe in this particular group for 3 months.

Conclusion

Under the conditions tested in this double-blind, placebo-controlled trial in postmenopausal women, 3.2 g CLA isomer blend (50:50% cis-9, trans-11: trans-10, cis-12 isomers) did not affect markers of bone metabolism and calcium.

References

1- Barret CE. Epidemiology and the menopause: A global overview. Int J Fertil 1993; 38:6-14.
2- NIH Concensus Statement, Osteoporosis prevention, diagnosis, and therapy, National Institutes of Health Concensus Development Conference Statement. 2000; 17 (2):1-36.
3- Dempster DW, Lindsay R: Pathogenesis of osteoporosis. Lancet 1996; 341:797-801.
4- Kazemian GH, Manafi AR, Najafi F, Najafi MA. Treatment of intertrochanteric fractures in elderly highrisk patients: Dynamic Hip Screw vs. External Fixation. Injury 2013, 10.1016/j.injury.2013.11.020
5- Scharbo DM. Hormone replacement therapy. Nurse Pract 1999; 21:1-13.
6- Groeneveld FP, Bareman FP. Determinants of first prescription of hormone replacement therapy. A follow up study among 1689 women aged 45-60 years. Maturitas 1994; 20:81-9.
7- Tucker KL, Chen H, Hannan MT. Bone mineral density and dietary patterns in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr 2002; 76:245–52.
8- Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem 2006; 17:789-810.
9- Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids 1997; 32:853– 8.
10- 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; 34:235–41.
11- Chung S, Brown JM, Sandberg MB, McIntosh M. Trans-10,cis-12 CLA increases adipocyte lipolysis and alters lipid droplet-associated proteins: role of mTOR and ERK signaling. J Lipid Res 2005; 46:885– 95.
12- Larsen TM, Toubro S, Astrup A. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. J Lipid Res 2003; 44:2234–41.
13- Steck SE, Chaleck AM, Miller P, Conway J, Austin GL, Hardin JW, et al. Conjugated linoleic acid supplementation for twelve weeks increase lean body mass in obese humans. J Nutr 2007; 137: 1188-93.
14- Gaullier JM, Halse J, Heivik HO, Heye K, Syvertsen C. Six month's supplementation with conjugated linoleic acid induces regional-specific fat mass decrease in overweight and obese. Br J Nutr 2007; 97: 550-60.
15- Laso N, Bruque E, Vidal J, Ros E, Arnaiz JA. Effects of milk supplementation with conjugated linoleic acid (isomers cis-9, trans-11 and trans-10, cis-12) on body composition and metabolic syndrome components. Br J Nutr 2007; 98:860-7.
16- Malpuech-Brugère C, Verboeket-van de Venne WP, Mensink RP, Arnal MA, Morio B, Brandolini M, et al. Effects of two conjugated linoleic acid isomers on body fat mass in overweight humans. Obes Res 2004; 12:591– 8.
17- 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; 130:2943–8.
18- Liew C, Schut HA, Chin SF, Pariza MW, Dashwood RH. Protection of conjugated linoleic acids against 2-amino-3-

methylimidazo [4, 5-f] quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. Carcinogenesis 1995; 16:3037– 43.
19- Belury MA. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. J Nutr 2002; 132:2995 –8.
20- Belury MA, Nickel KP, Bird CE, Wu Y. Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. Nutr Cancer 1996; 26:149– 57.
21- Ip C, Banni S, Angioni E. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J Nutr 1999; 129:2135–42.
22- Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 1994; 108:19– 25.
23- Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hyper cholesterolemic hamsters. Artery 1997; 22:266– 77.
24- Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. J Am Coll Nutr 2000; 19:472S– 7S.
25- Koba K, Akahoshi A, Yamasaki M. Dietary conjugated linoleic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. Lipids 2002; 37: 343–50.
26- Ryder JW, Portocarrero CP, Song XM. Isomer-specific anti diabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 2001; 50:1149– 57.
27- Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun 1998; 244:678–82.
28- Nugent AP, Roche HM, Noone EJ, Long A, Kelleher DK, Grbney MJ. The effect of conjugated linoleic acid supplementation on immune function in healthy volunteers. Eur J Clin Nutr 2005; 59: 742-50.
29- 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; 228:51– 8.
30- Yu Y, Correll PH, Vanden Heuvel JP. Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. Biochim Biophys Acta 2002; 1581:89– 99.
31- Iwakiri Y, Sampson DA, Allen KG. Suppression of cyclooxygenase- 2 and inducible nitric oxide synthase expression by conjugated linoleic acid in marine macrophages. Prostaglandins Leukot Essent Fatty Acids 2002; 67:435–43.
32- Najafi MR, Shaygannajad V, Mirpourian M, Gholamrezaei A. Vitamin B(12) Deficiency and Multiple Sclerosis; Is there Any Association? Int J Prev Med 2012; 3:286-9.
33- Fritsche JRR, Steinhart H. Formation, contents, and estimation of daily intake of conjugated linoleic acid isomers and trans-fatty acids in foods. Advances in Conjugated Linoleic Acid Research 1999; 1:378 – 96.
34- Platt I, Rao LG, EL-Sohemy A. Isomer-specific effects of conjugated linoleic acid on mineralized bone nodule formation from human osteoblast-like cells. Exp Biol Med 2007; 232:246-52.
35- Watkins BA, Shen CL, McMurtry JP, Xu H, Bain SD, Allen KG, et al. Dietary lipids modulate bone prostaglandin E2 production, insulin-like growth factor-1 concentrations and formation rate in chicks. J Nutr 1997; 127: 1084-91.
36- Watkins BA, Seifert MF. Conjugated linoleic acid and bone biology. J Am Coll Nutr 2000; 19:478S-486S.
37-Klein-Nulend J, Burger EH. Prostaglandins and bone. Euro Cal Tiss Soc Available at http://www.ectsoc.org/reviews/006_klei.htm accessed on October 2008.
38- Albertazzi P, Coupland K. Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention? Maturitas 2002; 42:13 –22.
39- Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000; 51:245–70.
40- Bruunsgaard H, Pedersen M, Pedersen BK. Aging and pro inflammatory cytokines. Curr Opin Hematol 2001; 8:131 – 6.
41- Ferrucci L, Corsi A, Lauretani F. The origins of age-related pro inflammatory state. Blood 2005; 105:2294 –9.
42- Morley JE, Baumgartner RN. Cytokine-related aging process. J Gerontol A Biol Sci Med Sci 2004; 59: M 924–9.
43- Goldring SR. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheumatology (Oxford) 2003; 42(Suppl 2):ii11–6.
44- Troen BR. Molecular mechanisms underlying osteoclast formation and activation. Exp Gerontol 2003; 38:605– 14.
45- Suda K, Udagawa N, Sato N. Suppression of osteoprotegrin expression by prostaglandin E2 is crucially involved in lipopolysaccharide- induced osteoclast formation. J Immunol 2004; 172: 2504– 10.
46- Okada Y, Lorenzo JA, Freeman AM. Prostaglandin G/H synthase-2 is required for maximal formation of osteoclast-like cells in culture. J Clin Invest 2000; 105:823–32.
47- Kelly O, Cusack S, Jewell C, Cashman KD. The effect of polyunsaturated fatty acids, including conjugated linoleic acid, on calcium absorption and bone metabolism and composition in young growing rats. Br J Nutr 2003; 90:743–50.
48- Rahman M, Bhattacharya A, Banu J, Fernandes G. Conjugated linoleic acid protects against age-associated bone loss in C57BL/6 female mice. J Nutr Biochemistry 2007; 18:467- 474.
49- Hur SJ, Park Y. Effect of conjugated linoleic acid on bone formation and rheumatoid arthritis. Eur J Pharmacology 2007; 569:16-24.
50- Weiler H, Austin S, Fitzpatrick-Wong Sh, Nitschmann E. Conjugated linoleic acid reduces parathyroid hormone in health and polycystic kidney disease in rats. Am J Clin Nutr 2004; 79: 1186s-9s.
51- Terpstra AHM. Effect of Conjugated linoleic acid on body composition and plasma lipids in humans. Am J Clin Nutr 2004; 79:352-61.