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Impact of conjugated linoleic acid on bone physiology: proposed mechanism involving inhibition of adipogenesis

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Abstract

Conjugated linoleic acid (CLA) supplementation decreases adipose mass and increases bone mass in mice. Recent clinical studies demonstrate a beneficial effect of CLA on reducing weight and adipose mass in humans. This article reviews possible biological mechanisms of action of CLA on bone metabolism, focusing on modulation of nuclear receptor peroxisome proliferator-activated receptor gamma activity to steer mesenchymal stem cell differentiation toward an adipose and away from an osteoblast lineage. Clinical studies of the effects of CLA on bone mass and clinical implications of the effects of CLA on bone health in humans are summarized and discussed.

Keywords

adipose; bone mineral density; conjugated linoleic acid; fatty acids; peroxisome proliferator-activated receptor gamma

Introduction

Osteoporosis is characterized by compromised bone strength, which increases the risk for fracture[1] and contributes to a considerable public health burden in the US population.[2] The current annual incidence of osteoporotic fracture is estimated at >2 million and the associated direct medical costs at $17 billion per year; these are projected to rise to 3 million fractures costing $25 billion per year in 2025.[3]

Conjugated linoleic acid (CLA) describes a group of positional and geometric isomers of the 18-carbon polyunsaturated fatty acid (PUFA) octadecadienoic acid, which contains two conjugated double bonds.[4] Dietary CLA is found naturally in meat and milk products from cattle, lamb, and goat. It is also available as a dietary supplement purported to cause weight loss. Among 28 different isomers of CLA, the major isomers of CLA found in dietary supplements are an equal ratio of cis-9, trans-11 (c9t11)-CLA (rumenic acid), and t10c12-CLA.[5] CLA has anticarcinogenic properties when used in chemically induced models of carcinogenesis in rodents.[6,7] Additionally, the t10c12 isomer of CLA reduces adipose mass in mice and humans, while the c9t11 isomer has anti-inflammatory properties.[8–12] Recent studies suggest t10c12-CLA induces retention of bone mineral density (BMD) in older

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mice.[13] Further, in growing mice fed a calcium-enriched diet, t10c12-CLA increased ash content as a measure of BMD.[14] The aim of this paper is to provide a review of primary literature supporting and opposing the proposal that CLA modulates bone physiology through a mechanism dependent on changes of peroxisome proliferator-activated receptor (PPAR)-γ-mediated induction of adipogenesis of mesenchymal cell differentiation to adipocytes in bone. Additional biological mechanisms and possible clinical implications will be discussed.

Conjugated Linoleic Acid Lowers Adipose Mass

CLA lowers adipose mass in several strains of growing male and female mice. The t10c12-CLA isomer is responsible for the adipose-lowering effects. Demonstrated in differentiated adipocytes, the mechanism of adipose-decreasing activity likely involves inhibition of heparinreleasable lipoprotein lipase, resulting in increased apoptosis of preadipocytes.[15–18] The t10c12-CLA isomer appears to inhibit differentiation to mature adipocytes via the downregulation of PPAR-γ.[19–21] Addition of a thiazolidinedione, ciglitazone, induced PPAR-γ mRNA and protein expression in a murine preadipocyte cell line, and cotreatment with t10c12-CLA abolished these effects.[20] In the same study, mice supplemented with CLA showed decreased gene expression of PPAR-γ in adipose tissue. Physiological concentrations (10–30 μmol/L) of t10c12-CLA decreased, whereas c9t11-CLA increased PPAR-γ expression and downstream target gene expression versus vehicle control in primary cultures of human preadipocytes; however, both isomers antagonized PPAR-γ ligand-dependent activation.[21] Inhibition of PPAR-γ expression and target gene expression by t10c12-CLA also occurs in murine and human adipocyte cultures.[22]

Until recently, the adipose-lowering effect of t10c12-CLA in humans was not demonstrated. Reasons for these negative data include lack of adequately powered studies and short durations of clinical interventions to show measurable changes of adipose mass. By 2002, a few studies of 6–12 months' duration demonstrated a dose-responsive lowering of adipose mass in trials involving men and women.[9] A meta-analysis predicted that for each 1 g of CLA consumed, a fat loss of 0.024 kg (0.05 lb) per week could be achieved.[12]

We recently tested the effect of 6.4 g per day of CLA (of which 3.2 g included the adipose-lowering t10c12-CLA) on alterations in adipose mass in obese women with type 2 diabetes. The study involved 55 postmenopausal women in a crossover double-blinded intervention in which the comparator oil was linoleic-acid-rich safflower oil. Supplementation with CLA lowered body fat over a 16-week period by over 1.5 kg in obese postmenopausal women with type 2 diabetes.[23] These data confirmed the earlier observation that t10c12-CLA effectively lowers adipose mass in obese humans.

Conjugated Linoleic Acid Alters Bone Mineral Content in Mice

Effects of CLA on bone ash or mineral content in animals vary, depending on the species investigated. Since skeletal bone serves as the major reservoir of minerals, bone ash serves as a valid surrogate measure for bone mass. The CLA-induced increase in bone ash content was observed in chicks[24] and mice,[25] but not in rats,[26–30] and was found variably in pigs.[31–33] When data from multiple CLA trials in mice were compiled, Park and Pariza[14] suggested an interaction of dietary calcium with the ability of CLA to increase bone mass. These findings are supported by a recent study demonstrating that CLA supplementation increases bone ash, but only in those animals fed a higher level of dietary calcium (1% versus 0.05% or 0.01%).[34] Further, the effect was due to the t10c10 rather than the c9t11 isomer.[34]

Supplementation of experimental diets with CLA for 14 weeks in young (2-month-old) male mice resulted in increased volumetric bone mineral content, bone area, and BMD of trabecular and cortical bone, despite decreases in body weight gain.[35] Notably, bone size increased and was accompanied by greater periosteal perimeter, endocortical perimeter, and cortical thickness. These changes in bone geometry induced by CLA suggest CLA can promote growth of a larger and thicker skeleton that is biomechanically stronger (Figure 1).[36] A similar 10-week feeding experiment in middle-aged (12-month-old) female mice showed that CLA increased volumetric BMD of trabecular and cortical bone.[13] CLA treatment in both young and middle-aged animals increased cortical thickness and cortical bone area due to increased periosteal bone, but in the middle-aged mice, some bone sites (e.g., femoral neck) showed a decrease in endocortical perimeter, suggesting decreased bone resorption at this bone surface. Exercised middle-aged mice also showed increases in bone mass and size, which was additive in combination with CLA.[13]

CLA has been shown to affect physiological markers of bone turnover. In middle-aged mice, CLA decreased the proinflammatory cytokines interleukin 6 and tumor necrosis factor-α, decreased the key soluble regulator of osteoclastogenesis, the receptor activator of NF-κβ ligand (RANKL), and decreased the bone resorption biomarker serum tartrate-resistant acid phosphatase 5b.[37] CLA supplementation in ovariectomized rats lowers a marker of bone resorption, urinary pyridinium crosslinks.[28] Like the isomer specificity of t10c12-CLA to lower adipose, this same isomer may be the bioactive isomer of CLA to alter bone. Supplementation with the t10c12-CLA, rather than the c9t11 isomer, increased whole-body ash content in mice[15] and increased osteoblast differentiation in mesenchymal stem cells.[38] In summary, animal models provide evidence for a beneficial effect of CLA on bone metabolism in aging.

Potential Mechanisms of Conjugated Linoleic Acid Action on Bone

The most compelling mechanism of action of CLA relates to effects on PPARs, a family of transcription factors within the nuclear hormone receptor superfamily, which also includes estrogen, vitamin D, thyroid, glucocorticoid, and retinoic acid receptors.[39] Four isoforms are described, PPAR-α, PPAR-β/δ, PPAR-γ1, and PPAR-γ2. Although many different tissues express PPAR-γ1 at low levels, mature adipocytes, including bone marrow adipocytes, express PPAR-γ2 at high levels. Human primary osteoblasts have been shown to express PPAR-γ and PPAR-β/δ.[40] Activity of PPAR as a transcription factor requires partnering as a heterodimer with a retinoid X receptor, with each receptor requiring a ligand for activation and interaction with specific coactivators and corepressors to bind cognate nuclear PPAR response elements.[41]

PPAR-γ and differentiation of mesenchymal stem cells

Adipocyte differentiation requires activation of PPAR-γ2. Within bone marrow tissue, differentiation of mesenchymal stem cells is steered toward an adipocyte versus an osteoblast lineage by activation of PPAR-γ2.[42,43] Alternatively, osteoblastogenic differentiation requires activation of the transcription factor, core-binding factor A1 (Cbfa1)/runt-related transcription factor (Runx)2.[44] Another transcription factor, CCAAT/enhancer-binding protein (C/EBP)β, maintains PPAR-γ expression during adipocytic differentiation, and PPAR-γ upregulates C/EBPα expression, which stimulates adipocyte gene expression.[45] PPAR-γ2 downregulates expression and activity of Cbfa1/Runx2,[44,46] thereby inhibiting osteoblastogenesis. PPAR-γ2 also suppresses other osteoblast-signaling pathways (Wnt, transforming growth factor-β/bone morphogenetic protein, and insulin growth factor-1) and transcriptional regulators (Osterix,Dlx5).[47] A number of transcription factors reciprocally regulate the cell fate of the multipotential bone marrow mesenchymal stem cell. The PPAR-γ1 isoform also promotes lineage commitment of hematopoietic stem cells toward osteoclast

development.[48] Thus, PPAR-γ upregulation within bone marrow would decrease bone formation, increase bone resorption, and increase marrow adiposity (Figure 2).

Whereas homozygous PPAR-γ deletion is lethal early in gestation (day 10–10.5), PPAR-γ haploinsufficient mice demonstrate a phenotype of high bone mass, increased osteoblastogenesis from bone marrow progenitors, and low bone marrow adiposity.[49] The inverse relationship of increased adiposity and diminished bone mass that commonly occurs in older-age individuals may be mediated by PPAR-γ activation steering lineage allocation of mesenchymal bone marrow stem cells toward adipogenesis and away from osteoblastogenesis.[50] The PPAR-γ agonist medication class, thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, increase tissue sensitivity to the action of insulin and are commonly used pharmacologic agents in the treatment of type 2 diabetes mellitus. Rosiglitazone treatment of a mesenchymal stem cell line transfected with PPAR-γ inhibited osteoblastogenesis and promoted adipogenesis.[51] In mice, rosiglitazone[52] and darglitazone[53] (at 20- and 100-fold increased potency relative to rosiglitazone and pioglitazone) decreased bone mass and bone formation rate and increased bone marrow fat mass. TZDs have been recently been associated with BMD loss in prospective cohort[54] and clinical trials.[55,56] Especially worrisome are recent clinical reports that TZD use is associated with fragility fractures of upper and lower extremities in observational studies,[57,58] clinical trials,[59,60] and a meta-analysis.[61]

Naturally occurring PPAR-γ ligands include arachidonic acid derivatives, various prostaglandins, and long-chain PUFAs. Some specific examples of highaffinity naturally occurring ligands for PPAR-γ include 15-deoxy-$\Delta^{12,14}$-prostaglandin J2,[62] 9(2)-HODE,[63] 15-hydroxy-eicosatetraenoic acid (15-HETE),[64] and 8-S-hydroxyeicosatetraenoic acid (8S-HETE). Several PUFAs and eicosanoids are promiscuous ligands that interact with two or more PPARs. Leukotriene B4 activates all PPARs, especially PPAR-α.[65] Both c9t11-CLA and t10c12-CLA isomers are potent ligands and activators of PPAR-α,[66] while both are modest activators of PPAR-γ.[67] Most PUFAs are moderately potent activators of PPAR-γ.

All three PPAR isoforms have been detected in cultures of human osteoclasts.[68] Specific agonists of PPAR isoforms as well as nonspecific PPAR activators (bezafibrate and fenofibrate) inhibited osteoclastogenesis. However, there were different effects of isoform-specific agonists on osteoclast function. PPAR-γ agonist (ciglitazone) inhibited, PPAR-β/δ agonist (L165041) stimulated, and PPAR-α agonist (GW9578) had a neutral effect on bone resorption.[68] PPAR-β/δ and PPAR-γ but not PPAR-α have been identified in human primary osteoblasts.[40]

Indirect effects of PPAR-γ on bone via adipokines

Of interest to bone physiology, activation of PPAR-γ by TZD may indirectly influence bone metabolism via adipokine secretion. A cross-sectional population study reported an inverse relationship between BMD and concentration of serum adiponectin, an adipocyte-derived hormone.[69] In contrast, adiponectin dose-dependently promoted proliferation and differentiation in cultured osteoblasts.[70] Serum concentrations of leptin, another adipocyte-derived hormone, reflect the mass of adipose in mice and in humans.[71] In leptin-deficient ob/ob mice and leptin-resistant db/db mice, bone mass of vertebral trabecular sites is higher while bone mass at femoral cortical and trabecular sites is lower than in nonmutated littermates fed similar diets.[72] Leptin inhibits bone formation via a hypothalamic pathway. Intracerebroventricular infusion of leptin in hypoleptinemic and wild-type mice activates hypothalamic sympathetic nerve fibers innervating osteoblasts via the B2-adrenergic receptor (ADRB2).[72] ADRB2 signaling in osteoblasts decreases bone formation and increases bone resorption, with the latter occurring via upregulation of RANKL.[73] In a parabiosis experiment, leptin administered via intracerebroventricular infusion did not cross

into systemic circulation and decreased bone mass only in the leptintreated animals.[74] The ADBR2-null mouse demonstrated increased trabecular bone mass at the vertebrae and distal femur and increased cortical bone at the mid femur.[75] CLA treatment decreased serum leptin concentration in mice[37] and lowered serum and white adipose tissue leptin concentration in rats.[76] Leptin can directly affect bone metabolism by steering differentiation of a bone marrow stromal cell line toward an osteoblast pathway without altering expression of the osteoblastogenic transcription factor Cbfa-1/Runx2 or the adipogenic transcription factor PPAR-γ.[77] Finally, bone marrow adipocytes produce adiponectin and leptin under the control of PPAR-γ[78] and osteoblasts express receptor for adiponectin[79] and leptin,[73] suggesting that bone remodeling may also be modulated locally.

Clinical Studies of Conjugated Linoleic Acid on Bone

A search of medical literature was undertaken using PubMed with the following search details: “conjugated”[All Fields] AND (“linoleic acid”[MeSH Terms] OR (“linoleic”[All Fields] AND “acid”[All Fields]) OR “linoleic acid”[All Fields]) AND (“bone and bones” [MeSH Terms] OR (“bone”[All Fields] AND “bones”[All Fields]) OR “bone and bones” [All Fields] OR “bone”[All Fields]). Of the 52 papers identified, only the in vivo clinical studies in human subjects were included in this review. There were four studies with bone turnover biomarkers and five studies with dual X-ray absorptiometry (DXA) data. The latter group is summarized in Table 1.

In an 8-week intervention study in 60 men, supplementation with CLA versus placebo did not affect bone formation biomarkers (serum osteocalcin and bonespecific alkaline phosphatase), bone resorption biomarkers (serum C-telopeptide, urinary N-telopeptide, urinary pyridinoline, and urinary deoxypuridinoline), serum calcium concentrations, or urinary calcium concentrations.[80] Likewise, other short-term studies involving 1–6 months of strength training plus CLA versus placebo did not reveal changes in bone turnover markers.[81–83]

It is well established that decreasing body weight is associated with declining BMD in clinical trials of weight loss[84,85] and in prospective epidemiological studies.[86,87] Although CLA reduces body weight by decreasing fat mass, bone mass is not compromised, and there is some evidence to support a beneficial effect of CLA on bone. Brownbill et al.[88] assessed the relationship between dietary CLA consumption (c9t11-CLA) and BMD in a cross-sectional study of 136 subjects. Dietary CLA assessed using 3-day diet records was positively associated with BMD at the Ward's triangle component of the proximal hip. BMD at the distal forearm was greater in subjects with a higher CLA intake. This is the only clinical study in postmenopausal women (mean age, 68.6 years; mean BMI, 26) using site-specific bone densitometry. The largest and longest human intervention study of CLA with skeletal outcomes data is reported by Gaullier et al.,[89] who randomized 180 men and women to CLA-free fatty acid (CLA-FFA), CLA-triacylglycerol (CLA-TG, 3.4 g of equal parts c9t11 and t10c12), or placebo. The CLA groups exhibited a 6.9–8.7% decrease in body fat mass versus placebo. During a follow-up in a 1-year open-label extension study in which all subjects took CLA-TG supplements, decrements in body fat mass previously observed in the CLA groups were maintained, and the placebo group (switching to CLA) experienced a comparable decrease in body fat mass.[90] The CLA-TG group and the placebo group showed no changes in bone mineral mass (BMM) by body composition DXA at 0, 1, and 2 years. The CLA-FFA group showed a small 1.4% decrease in BMM from baseline to year 1, which returned to baseline by the second year. In a subsequent clinical trial to determine which body compartments accounted for loss in body fat, CLA supplementation (3.4 g of active isomer) led to a decrease in fat mass, mainly in the leg; however, total body bone mineral content did not change.[91] Assessment of specific skeletal sites was not performed. Recently,

CLA supplementation (3 g/day of 80% t10c12-CLA and c9t11-CLA in equal proportion) versus placebo in overweight and obese 6- to 10-year-old children attenuated increases in BMI and body fat but decreased accrual of total body bone mineral content (CLA + 0.05 ± 0.03 kg versus placebo 0.07 ± 0.03 kg, $P = 0.04$).[92] Notably, this study did not assess bone size, which was increased in a preclinical study[13] Further study using 3D assessment of bone geometry would be needed to clarify this potential safety issue in the growing human skeleton exposed to CLA supplementation. Since these interventional studies were primarily designed to evaluate the effect of CLA on weight loss, the effect on changes in bone mass are inconclusive. Of note, in these clinical trials, assessment of bone mass is limited to measures of total body bone mass using body composition DXA.

In summary, CLA maintains an overall neutral effect on bone mass despite inducing loss of adiposity and body weight. Total body BMM rather than BMD was reported, and site-specific measurements of BMD such as lumbar spine, proximal hip, and distal forearm have not been reported, which limits interpretation of the clinical effects of CLA on bone outcomes. Menopausal status of women has not reported in previous studies but could contribute to confounding issues with interpreting the data from previous studies. It could be presumed that many subjects in previous studies were premenopausal, since the mean age was 44 to 48 years.

Conclusion

Preclinical data support a positive effect of CLA on bone health, whereas clinical data in human subjects have yet to show convincing evidence of a benefit. If CLA-induced decrement in adipose mass and weight is taken into account, a decrease in BMD might be expected; in contrast, maintenance of BMD with CLA supplementation has been observed.[89,90,91] Future clinical trials in specific populations, e.g., in older women to prevent postmenopausal bone loss, are necessary to elucidate the proper role of CLA supplementation to enhance skeletal health.

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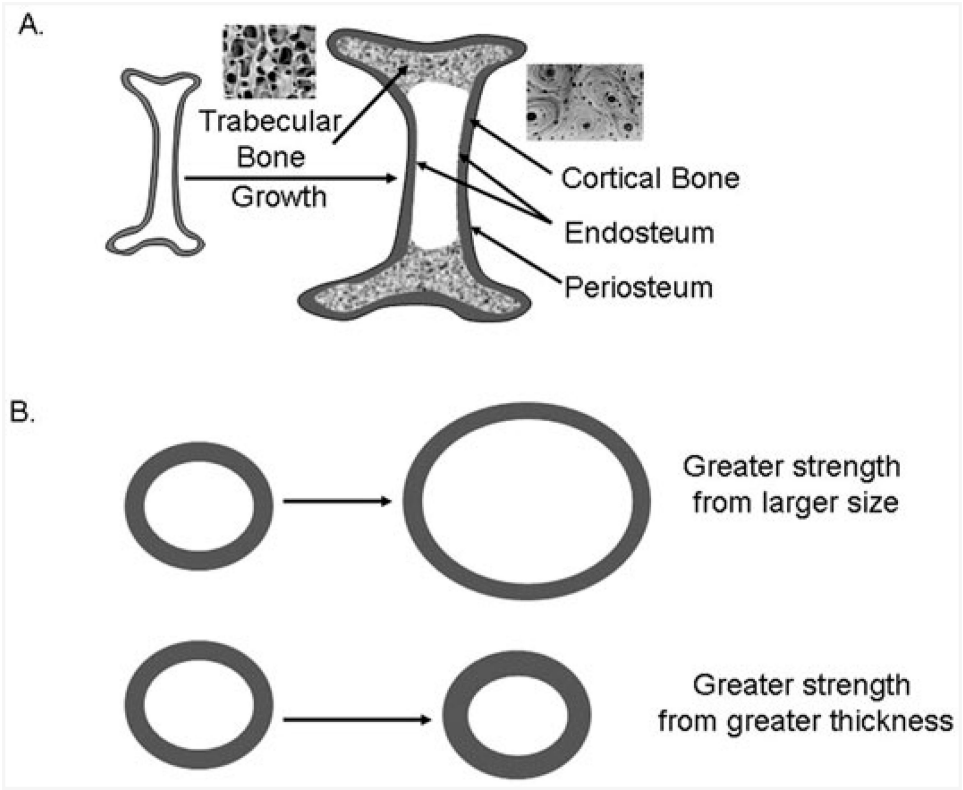

Figure 1. A. Schematic longitudinal section of a long bone showing outer cortical bone and inner trabecular bone tissue

Cortical bone is denser and is organized in concentric lamellar structures called osteons. Trabecular bone is more porous and is organized as interconnected plates and struts. Representative light microscopy images of human trabecular and cortical bone are shown. Bone growth involves resorption at the endosteal surface and new bone apposition at the periosteal surface, producing greater cortical bone diameters and thickness. **B. Schematic cross-section of a long bone.** Cortical bone size is an important determinant of bone strength. Bone strength is increased by a greater cortical diameter or greater cortical thickness.

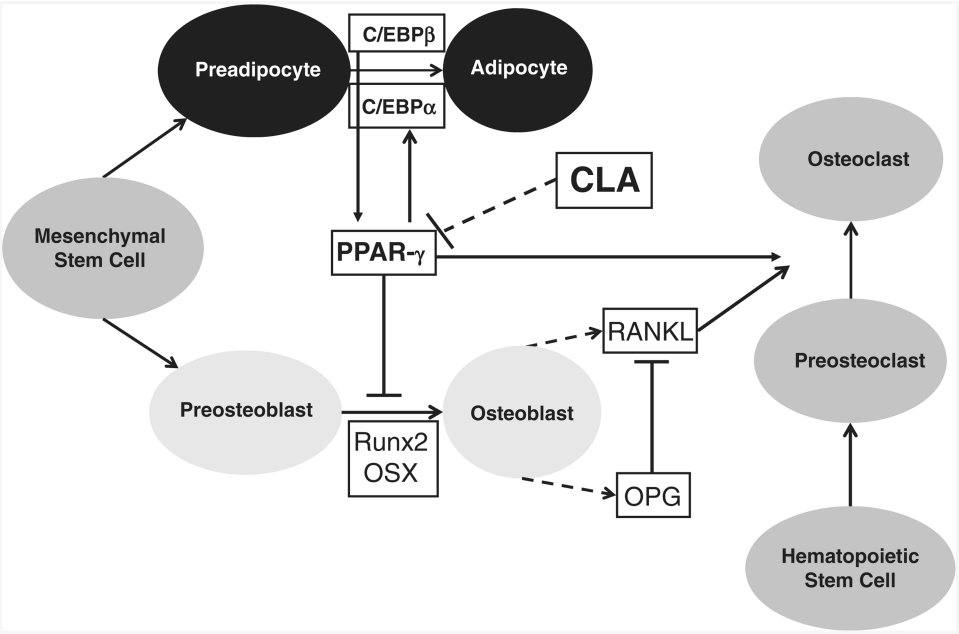

Figure 2. Possible differentiation of mesenchymal stem cells toward an adipocytic or osteoblastic lineage

Activation of transcription factor PPAR-γ and C/EBP promotes an adipocyte cell fate, whereas activation of transcription factor Cbfa1/Runx2 steers lineage allocation toward an osteoblast fate. PPAR-γ stimulates C/EBPα, and C/EBPβ induces PPAR-γ. PPAR-γ also inhibits Run×2 and promotes osteoclast formation from hematopoietic stem cells. Osteoblast-derived RANKL induces osteoclastogenesis, and OPG inhibits RANKL. *Abbreviations*: PPAR-γ, peroxisome proliferator-activated receptor gamma; C/EBP, CCAAT/enhancer-binding proteins; Cbfa1, core-binding factor A1; Runx2, runt-related transcription factor 2; RANKL, receptor activator of nuclear transcription factor κβ ligand; OPG, osteoprotegerin.

Table 1

Clinical data on the effects of conjugated linoleic acids (CLA) on bone.

Study	Study population	CLA formulation	Study type/duration	Outcome
Brownbill et al. (2005)[88]	136 postmenopausal women; 68.6 years; BMI 26.0	Dietary CLA by 3-day food diary	Cross-sectional	Dietary CLA correlated with hip BMD
Gaullier et al. (2004)[89] and (2005)[90]	31 men, 149 women; 44.5–48.0 years; BMI 27.7–28.3 (extension study, 134 subjects)	CLA-FFA: 4.5 g of 80% CLA (3.46 g CLA, 39% c9t11, 41% t10c12); CLA-TG: 4.5 g of 76% CLA (3.4 g CLA, 38% c9, t11, 38% t10, c12); Placebo: 4.5 g olive oil	Placebo-controlled randomized trial, 12 months with 12-month open-label extension	Total body bone mineral mass decreased in the CLA-FFA group at first year but returned to baseline at second year
Gaullier (2007)[91]	21 men, 84 women; mean age 45.8–48.7 years; BMI 32–35	CLA: 4.5 g of 80% CLA (3.4 g CLA, 37.5% c9t11, 38% t10c12); Placebo: 4.5 g olive oil	Placebo-controlled randomized trial, 6 months	Total body bone mineral content did not change between or within groups
Racine (2010)[92]	31 boys, 22 girls; ages 6–10 years; BMI >85th percentile	Clarinol™ 3.0 g (80% CLA, 50% c9t11, 50% t10c12); Placebo: sunflower oil	Placebo-controlled randomized trial, 7 months	Total body bone mineral content accrual was decreased in CLA group

Abbreviations: CLA-FFA, CLA-free fatty acid; CLA-TG, CLA triacylglycerol; c9t11, cis-9, trans-11 CLA; t10c12, t10c12 CLA.