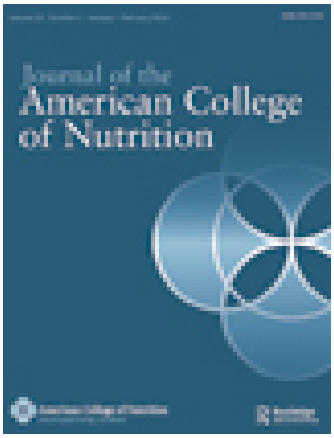

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# Conjugated Linoleic Acid and Bone Biology

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# Conjugated Linoleic Acid and Bone Biology

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**Key words: bone, cartilage, conjugated linoleic acid, rat**

Osteoporosis, osteoarthritis and inflammatory joint disease afflict millions of people worldwide. Inflammatory cytokines inhibit chondrocyte proliferation and induce cartilage degradation for which part of the response is mediated by $PGE_2$. Excess production of $PGE_2$ is linked to osteoporosis and arthritis and is associated with bone and proteoglycan loss. $PGE_2$ also influences the IGF-I/IGFBP axis to facilitate bone and cartilage formation. Recent investigations with growing rats given butter fat and supplements of CLA demonstrated an increased rate of bone formation and reduced *ex vivo* bone $PGE_2$ production, respectively. Furthermore, the supplements of CLA isomers resulted in their enrichment in lipids of various bone compartments of animals. The effects of CLA on bone biology in rats (IGF action and cytokines) appear to be dependent on the level of n-6 and n-3 fatty acids in the diet; however, these studies generally showed that CLA decreased *ex vivo* bone $PGE_2$ production and in osteoblast-like cultures. Anti-inflammatory diets, including nutraceutical applications of CLA, may be beneficial in moderating cyclooygenase 2 (COX-2) activity or expression (influencing $PGE_2$ biosynthesis) and might help to reduce rheumatoid arthritis (secondary osteoporosis). This review summarizes findings of CLA on bone modeling in rats and effects on cellular functions of osteoblasts and chondrocytes. These experiments indicate that CLA isomers possess anti-inflammatory activity in bone by moderating prostanoid formation.

**Key teaching points:**

- Dietary sources of conjugated linoleic acids accumulate in lipids of bone tissues.
- Conjugated linoleic acids alter the production and action of localized factors in bone to influence bone modeling.
- Studies on individual isomers of conjugated linoleic acid in bone and cancer research must be done.



## INTRODUCTION

Bone is a multifunctional organ that consists of a structural framework of mineralized matrix and contains heterogeneous populations of chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes and hemopoietic cells. This milieu of cells produces a variety of biological regulators that control local bone metabolism. Systemic calcitropic hormones [parathyroid hormone (PTH), estrogen, and $1,25(OH)_2$vitamin$D_3$] and autocrine and paracrine factors, including prostaglandins, cytokines and growth factors orchestrate the cellular activities of bone growth to increase the length and diameter of and properly shape long bones in children. Bone grows in size and shape through the collective activities of cells that produce, mineralize and resorb bone matrix. Bone matrix is produced and mineralized through the activity of the osteoblasts; in contrast, bone matrix resorption is accomplished by specialized multinucleated cells called osteoclasts [1]. The combined and cooperative activities of osteoblasts and osteoclasts result in a bone architecture that provides mechanical support and protection for the body. In addition, bone serves as a vital reservoir of minerals, principally calcium and phosphorus, necessary for maintaining normal cellular, neurologic and vascular activities of the body. Osteoblasts are mononucleated bone-forming cells that originate locally from mesenchymal stem cells. Osteoblasts are recruited to a site of bone formation where they actively synthesize and secrete an organic bone matrix called "osteoid," which is composed chiefly of type I collagen and other noncollagenous proteins. Following its formation, osteoid normally

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undergoes rapid mineralization with hydroxyapatite. In addition to synthesizing bone matrix, osteoblasts maintain a high alkaline phosphatase activity and produce numerous regulatory factors including prostaglandins, cytokines and growth factors. These locally produced compounds are reported to stimulate bone formation and/or bone resorption [2–5].

Osteoclasts are large multinucleated bone-resorbing cells. They form at skeletal sites from the fusion of mononuclear hemopoietic precursors that arrive via the vasculature. During bone resorption, osteoclasts produce and release lysosomal enzymes, hydrogen protons and free radicals into a confined space or resorptive compartment next to bone that dissolve the mineral and degrade bone matrix [6]. Activated osteoclasts are in contact with mineralized surfaces and produce distinctive resorptive cavities called Howship's lacunae. Thus, bone cells are under the regulatory control of systemic and local factors that modulate their activities and influence bone modeling and remodeling processes.

## BONE GROWTH

Bone is a dynamic connective tissue consisting of living cells embedded within or lining surfaces of a mineralized organic matrix. Bone provides mechanical support for the body and, through attachment of muscles, allows for locomotive movement through space. Furthermore, skeletal tissue protects vital organs and serves as a metabolic reservoir of calcium and phosphate for the body. Anatomically, the bones of the skeleton can be classified according to their individual shapes: flat (bones forming the roof of the skull, scapula and ilium), short (carpal and tarsal bones), irregular (vertebrae) and long (humerus, radius, ulna, femur and tibia).

All bone is derived from mesenchymal tissue; however, two different histogenetic processes exist for producing bone: one direct and another indirect through a temporary cartilage model. Intramembranous ossification occurs within presumptive flat bones by direct differentiation of mesenchymal cells into osteogenic cells. These osteoblasts deposit organic matrix within their embryonic connective tissue membrane that becomes mineralized. Long bones are formed by endochondral ossification, a process where embryonic mesenchymal cells differentiate into chondroblasts and chondrocytes that secrete hyaline cartilage matrix and produce a cartilage model of the future bone. During maturation of this tissue, chondrocytes within the future shaft (diaphysis) and ends (epiphyses) of the bone hypertrophy and the surrounding matrix undergoes mineralization. Invasion of these regions by the vasculature results in diaphyseal and epiphyseal centers of ossification. Mineralized cartilage matrix is removed by chondroclasts and replaced with bone by newly arrived osteogenic cells. These respective centers grow and expand toward one another, but remain temporarily separate by a bar of cartilage, the epiphyseal growth plate. This plate of cartilage, interposed between epiphyseal and metaphyseal regions of a bone, provides the means for bones to grow in length. In this process, chondrocyte proliferation, matrix production, mineralization and vascular invasion are balanced with removal of mineralized trabeculae from the metaphyseal side of the growth plate through osteoclastic activity. These primary trabeculae of mineralized cartilage enclosed by bone are progressively replaced by secondary trabeculae of bone produced by osteoblasts. This trabecular or cancellous bone constitutes a meshwork of tissue at the ends of long bones, the surfaces lined by bone cells and whose intertrabecular spaces are filled with hemopoietic tissue. Trabecular bone is mainly involved with metabolic functions. Dense or cortical bone completely encases bones and is especially thick within the diaphyses or shafts of long bones. The diameters of bones increase via intramembranous ossification through apposition of bone matrix by osteoblasts located within the periosteum. Cortical bone serves primarily mechanical and protective functions.

## BONE MODELING AND REMODELING

Bone modeling describes the continuous changes in bone shape, length and width throughout the growth of an individual until skeletal maturity is reached. In contrast to bone remodeling, bone modeling lacks local coupling of resorption with bone formation on bone surfaces. Resorption and formation occur on separate surfaces (periosteal or cortico-endosteal); therefore, surface activation in modeling bone may be followed by either resorption or formation (Table 1).

The skeletal morphology of the adult represents a sophisticated compromise between structural obligation and metabolic responsibility, serving the individual in support and locomotion

**Table 1.** Comparison of Bone Modeling and Remodeling Activities

| | Bone Modeling | Bone Remodeling |
|---|---|---|
| Local Coupling | Formation and resorption are *not* coupled | Formation and resorption are coupled |
| Timing & Sequence of Activity | F and RS are continuous and occur on separate surfaces | Cyclical: A[1]-RS[2]-RV[3]-F[4]; formation always follows resorption |
| Extent of Surface Activity | 100% of surfaces are active | 20% of surfaces are active |
| Anatomical Objectives | Gain in skeletal mass and change in skeletal morphology | Skeletal maintenance |

[1] A=activation, [2] RS=resorption, [3] RV=reversal, [4] F=formation

while actively participating in the regulation of calcium homeostasis. This compromise is accomplished through the individual's genetic potential for growth and intricate interactions between nutrition, metabolism, endocrine factors and transcription factors. Hormones and certain nutrients modulate the autocrine and paracrine influences (actions of prostaglandins, cytokines and growth factors) on cells and are responsible for the maintenance of bone mass and architecture. In the adult skeleton, the coordination of bone resorbing and bone forming activities is termed the "bone remodeling cycle".

The regulation of bone remodeling, and its corresponding role in the maintenance of adult bone mass, is distinctly different from the processes that control skeletal growth and modeling in the young (Table 1). As the name implies, modeling is responsible for creating bone shape. Modeling of bone is an adaptive process, providing order and specificity to the generalized increase in bone mass which accompanies body growth.

Bone remodeling involves the removal and internal restructuring of previously existing bone and is responsible for the maintenance of tissue mass and architecture in the adult skeleton [7]. Chemical and/or electrical stimuli activate local bone cell populations and coordinate their activities in removing and replacing discrete "packets" of bone at skeletal sites. These organized groups of cells are called "basic multicellular units" or BMU [8] which function within a defined remodeling cycle. Osteoblasts and osteoclasts are important members of the BMU.

The cellular interactions occurring within a remodeling cycle are divided into four main events; activation, resorption, reversal, and formation (Table 1) [9]. The remodeling cycle begins when a non-remodeling quiescent bone surface becomes "activated." The signals effecting activation are not fully understood, but it is believed that the bone lining cells covering inactive surfaces initiate this event. It is during this activation event that osteoclasts attach to the bone surface and resorb bone. This marks the resorption phase and results in the release of bone calcium. As the period of bone resorption subsides, the reversal phase represents a period of transition when the bone surface is repopulated with newly formed osteoblasts.

The formation phase begins as osteoblasts commence deposition of new bone matrix (osteoid), which subsequently becomes mineralized. This phase, and the bone remodeling cycle, is complete when the osteoblasts refill the cavity created during the resorption phase. These events of resorption and formation are coupled such that resorption is always followed by bone formation. It is hypothesized that a reservoir of growth factors and cytokines, previously produced by osteoblasts and incorporated into the bone matrix, become available locally as autocrine/paracrine factors during osteoclastic bone resorption. These factors direct the proliferation, differentiation and recruitment of new osteoblasts to the remodeling site and, thus, regulate the remodeling cycle [2–4] (Table 1).

## LOCAL REGULATION OF BONE METABOLISM

Bone formation and bone resorption are regulated by systemic hormones and local factors produced in bone [2–4,10]. Systemic hormones involved in stimulating bone formation include insulin, growth hormone [11] and estrogen [12], while those involved in stimulating bone resorption include 1,25-$(OH)_2$vitamin$D_3$ [13], PTH [14], thyroid hormone [15] and glucocorticoids [16]. In addition, calcitonin [17] inhibits bone resorption.

Even though several localized compounds act on bone cells, the prostaglandins (PG) seem to be the principle mediators of bone cell function since their biosynthesis and release from bone cells [18] and associated tissues can be induced by several cytokines as well as systemic factors [19]. $PGE_2$, which is synthesized from arachidonic acid, is a potent stimulator of bone resorption and is the primary PG affecting bone metabolism. The PG also influence insulin-like growth factors (IGFs), which are major bone-derived growth factors [2,20–22]. Once secreted and deposited in bone matrix, the IGFs are released during osteoclastic bone resorptive activity, acting in an autocrine or paracrine fashion to stimulate new bone-cell formation and matrix production. Thus, IGFs, in concert with other bone growth factors, play an essential role in the efficiency with which bone formation is coupled to bone resorption. The relationship between PGs and IGFs is important to the maintenance of skeletal mass during aging, as well as being vital in optimizing acquisition of skeletal mass during critical stages of skeletal growth and development (Table 2).

## CONJUGATED LINOLEIC ACIDS

Conjugated linoleic acid (CLA) is the name given to describe a group of positional and geometric fatty acid isomers of octadecadienoic acid. The double bonds in CLAs are conjugated, that is, not separated by a methylene group as in linoleic acid. Furthermore, CLAs will not substitute for linoleic acid as an essential fatty acid. CLAs occur naturally in ruminant food

**Table 2.** Responses of Autocrine and Paracrine Factors in Bone[1]

| Responses Observed in Bone | Cytokines, Eicosanoids, or Peptide Growth Factors[2] |
|---|---|
| Bone Formation or Matrix Production | IGF, $PGE_2$, TGF-$\beta$ |
| Bone Resorption | EGF, FGF, IL, LT, PDGF, TGF-$\alpha$, TNF-$\alpha$ |
| Collagen Synthesis | FGF, IGF, TGF-$\beta$ |

[1] Adapted from referenced [2–5,23]. [2] Epidermal growth factor=EGF; Fibroblast growth factor=FGF; Interleukin=IL; Insulin-like growth factor=IGF; Leukotriene=LT; Platelet-derived growth factor=PDGF; Prostaglandin E=$PGE_2$; Transforming growth factor=TGF-$\alpha$, TGF-$\beta$; Tumor necrosis factor=TNF-$\alpha$.

products (beef, lamb and dairy) because of the process of bacterial biohydrogenation of linoleic acid in the rumen [24]. The growing body of literature on CLA suggests that these isomeric conjugated fatty acids possess potent anticancer activity in mammary gland of rats [25,26], and reduce proliferation of human breast cancer cell lines in culture [27] and in SCID mice [28].

The positional isomers of CLA include Δ-7,Δ-9; Δ-8,Δ-10; Δ-9,Δ-11; Δ-10,Δ-12; Δ-11,Δ-13 and Δ-12,Δ-14 conjugated octadecadienoic acids (counting from the carboxyl end of the molecule). Each of the aforementioned positional conjugated diene isomers can occur in the following geometric configurations: *cis*-,*trans*-; *trans*-,*cis*-; *cis*-,*cis*- and *trans*-, *trans*- [29]. The most common CLA isomer found in food products is *cis*-9,*trans*-11-octadecadienoic acid [24]. The proposed name for this isomer of CLA is rumenic acid [30].

The unique biological actions of CLA include anticarcinogenic, antiatherosclerotic, antioxidative, immunomodulative and antibacterial. One of the earliest experiments on CLA indicated that these fatty acids, isolated from extracts of grilled ground beef, exhibited anticarcinogenic activity against chemically induced skin cancer in mice [31]. The CLA isomers are potent anticancer nutrients for epidermal and mammary tumors in rodents, and recent experiments on prostate cancer cell lines demonstrated that CLA isomers incorporate into cell lipids and, compared to linoleic acid, decrease cell proliferation [32].

The mechanism of CLA action on cell function is not well described; however, we have shown in rats that these fatty acids reduce *ex vivo* $PGE_2$ production in bone organ culture [33], serum IGF-I binding proteins [34] and basal and lipopolysaccharide-induced levels of IL-6 by resident peritoneal macrophages [35]. Other investigators have reported a significant reduction in serum $PGE_2$ [36,37] and splenic $LTB_4$ in rats given CLA [37].

## DIETARY LIPID EFFECTS ON BONE AND CARTILAGE METABOLISM

It is well documented that lipids play an important role in skeletal biology and bone health. For example, phospholipids facilitate cartilage mineralization in growth plate [38], and signals from biomechanical forces are mediated by PGs [19]. The PGs also aid in regulating anabolic factors, including IGFs [2], to support bone formation. Emerging evidence from human and animal research supports the hypothesis that dietary lipids influence bone modeling and remodeling. Epidemiological studies indicate that dietary fat intake is associated with reduced risk of vertebral and femoral fractures in adults and saturated fat intake caused an increase in bone density in children [39]. Recent investigations with chicks and rats revealed that PUFAs and CLA affect histomorphometric measurements of bone modeling [34,40,41].

Dietary fat may influence bone metabolism by altering PG biosynthesis [34,40,41]. The PGs, locally produced from 20-carbon essential fatty acid precursors [20:4(n-6) and 20:5(n-3)] in osteogenic cells, regulate both bone formation and bone resorption [19]. Recent animal studies on bone modeling support the relationship between dietary PUFA, PGs and bone metabolism. Watkins *et al.* reported that dietary lipids (n-3 fatty acids and CLA) modulated the production of $PGE_2$, altered the concentration of IGF-I in bone tissues and led to increased or decreased bone formation rates in growing chicks and rats [34,40]. In these experiments, rats given a supplement of CLA showed a decrease in the rate of bone formation, suggesting a down-regulating effect on osteoblastic activity.

The $PGE_2$ produced by osteoblasts may stimulate IGF-I synthesis or affect its action to support anabolic responses in bone [22]. Studies with dairy fats revealed that butter fat (source of natural CLA isomers) blended with corn oil reduced *ex vivo* bone $PGE_2$, elevated bone IGF-I concentration and increased bone formation rates in animals nearly 60% compared to those given diets higher in n-6 fatty acids [41]. The responses observed in bone tissue suggest that moderating the action of n-6 fatty acids (linoleic acid) with n-3 fatty acids or CLA can benefit bone modeling. Others have reported health benefits attributed to moderating the dietary intake of n-6 fatty acids. For example, research on heart disease and dietary lipids shows a positive association between linoleic acid concentrations in adipose tissue and coronary heart disease [42] and for decreasing the intake of PUFA to reduce oxidative modification of lipoprotein [43].

The studies on PUFA and bone modeling in animals suggest that dietary intakes of different PUFA families and CLA can affect bone modeling directly (by moderating PG biosynthesis) or indirectly (via IGFs). The anabolic effects of $PGE_2$ may occur through stimulation of osteoblast endogenous IGF-I production [4] or by increasing bone cell responsiveness to IGF-I. Dietary fatty acids, depending upon the type (n-3 or CLA) and amount ingested, may, therefore, up-regulate or down-regulate IGF-I production in bone via their ability to modulate local concentrations of $PGE_2$ [34,40,41,44]. The relationships between dietary PUFA and bone metabolism offer many promising opportunities for further biochemical and molecular research on local factors controlling bone cell function. The dietary factors that appear to influence bone cell activity to stimulate bone formation and bone resorption are illustrated in Fig. 1.

Investigations with PUFA and epiphyseal (growth) cartilage and chondrocytes also indicate that dietary lipids affect cartilage metabolism to influence bone modeling. Experiments on growth cartilage demonstrated that this tissue selectively accumulates dietary fatty acids [46,47]. These results suggest that chondrocytes are either sensitive to excess n-6 fatty acids or to an overproduction of $PGE_2$ [48]. Growth cartilage in children and young animals contains small amounts of n-6 fatty acids, but a relatively high concentration of 20:3(n-9) (Mead acid) [49]. The concentration of Mead acid is not reduced in growth

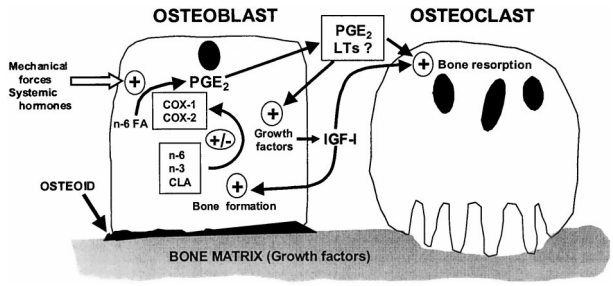


**Fig. 1.** Observed effects of dietary fatty acids and related compounds on osteoblastic and osteoclastic activity in bone [44]. Excessive biosynthesis of $PGE_2$ may depress bone formation and lead to increased bone resorption. Altering the production of eicosanoids (PGE and LTB) appears to optimize formation by osteoblasts perhaps by influencing IGF-I production and action [45].

cartilage of growing animals given diets adequate or enriched in linoleic acid (n-6) [46]. Furthermore, supplementation of growth cartilage chondrocytes with linoleic or arachidonic acids depressed collagen synthesis [48], but these cells showed greater collagen synthesis when enriched with n-3 fatty acids or CLA [50,51].

In our laboratory, primary cultures of avian epiphyseal chondrocytes were studied to assess the effects of CLA (a mixture of CLA positional and geometric isomers) on fatty acid metabolism, collagen synthesis and $PGE_2$ production [52]. In a series of experiments, chondrocytes were enriched with CLA and linoleic acid (LA) (0, 50, 100 and 200 $\mu$mol/L) or arachidonic acid (AA) and eicosapentaenoic acid (EPA) (0 and 50 $\mu$mol/L). Chondrocytes enriched with CLA contained *cis*-9, *trans*-11 and *trans*-10, *cis*-12 18:2 as the primary CLA isomers. CLA decreased the concentrations of 16:1 and 18:1 in chondrocytes compared to the LA treated cells. Enrichment of chondrocytes with CLA and LA affected collagen synthesis in a dose dependent fashion. The LA [48] and AA treatments reduced collagen synthesis as previously observed, but CLA and EPA appeared to stimulate its synthesis. Chondrocyte production of $PGE_2$ was reduced by CLA and EPA treatments, while LA and AA increased $PGE_2$ relative to the no fatty acid enrichment. These experiments suggest that CLA may positively influence growth plate cartilage function in the young and may reduce production of inflammatory $PGE_2$ in the adult.

## DIETARY CONJUGATED LINOLEIC ACIDS INFLUENCE BONE MODELING

Watkins *et al.* recently reported [41] that butter fat led to a higher rate of bone formation in chicks compared with those given diets containing higher amounts of n-6 fatty acids (linoleic). The greater rate of bone formation in the butter-fed chicks was associated with reduced bone concentration of arachidonic acid and *ex vivo* $PGE_2$ production and higher serum hexosamines and IGF-I in bone [41]. Since the beneficial effects of butter fat on bone might be attributed to its CLA content (milk fat contained 1.5% CLA), studies with these fatty acids were conducted in rats.

In our investigations with rats, dietary CLA led to differences in CLA enrichment of various organs and tissues; brain exhibited the lowest concentration of isomers, but bone tissues (periosteum and marrow) contained the highest [33]. Moreover, CLA altered the fatty acid composition of rat tissues, reducing 18:1 in liver, skeletal muscle, heart and bone marrow and periosteum [33]. In a subsequent study with rats, feeding 0.5% CLA resulted in total CLA concentrations ranging from 0.27% to 0.43% in the polar lipid fraction and from 2.02% to 3.37% in the neutral lipids in liver, bone marrow and bone periosteum. Our observation that CLA accumulated at a higher concentration in neutral lipids compared with polar lipids is consistent with that of Ip *et al.* [53] for rat mammary gland. In rats, the *trans*-10, *cis*-12 18:2 isomer was incorporated into the phospholipid fraction of tissue lipid extracts much like that for the *cis*-9, *trans*-11 isomer. The ratio of *cis*-9, *trans*-11/*trans*-10, *cis*-12 roughly reflected the isomeric distribution of these CLA isomers in the diet given to rats. Furthermore, the *cis*-9, *trans*-11 isomer of CLA was preferentially incorporated into rat membrane phospholipid [33].

It is hypothesized that CLA depresses arachidonate-derived eicosanoid biosynthesis based on reduced *ex vivo* $PGE_2$ production in rat bone organ culture and liver homogenate [34]. The reduction in $PGE_2$ by CLA might be explained as a competitive inhibition of n-6 PUFA elongation that results in lowered substrate availability for cyclooxygenase. Although there was a trend of reduced arachidonic acid concentration in bone tissues, the dramatic decrease in *ex vivo* $PGE_2$ production in bone organ culture could not be explained entirely by a lack of substrate. Bone biosynthesis of $PGE_2$ (cells of the osteoblast lineage) is highly regulated [54,55]. A primary step for the regulation of PG formation is at the level of the enzyme cyclooxygenase (COX, also called prostaglandin G/H synthase). Recently, investigators observed that fatty acids modulated the expression and activity of this key enzyme. For example, Nanji *et al.* [56] showed that saturated fat reduced peroxidation and decreased the levels of COX-2, the inducible form of COX, in rat liver. In a rat feeding study on colon tumorigenesis, a high-fat corn oil diet [rich in (n-6) fatty acids] up-regulated COX-2 expression, but a high-fat fish oil diet [rich in (n-3) fatty acids] inhibited it; however, expression of COX-1, the constitutive enzyme, was not affected [57]. We speculate that CLA may influence $PGE_2$ production through the COX enzyme system [34], more likely on COX-2, to affect bone (Fig. 2). Potentially, CLA may alter COX-2 action/expression to influence PTH [54] and growth factor [58] PG-dependent osteoclastic bone resorption, PGE receptor-mediated [55,59] actions on bone cells and cytokine-induced extracellular release of $PGE_2$ [60] by osteoblasts.

Other possible mechanisms of action for CLA include reduced desaturation/elongation of linoleic acid and inhibition of

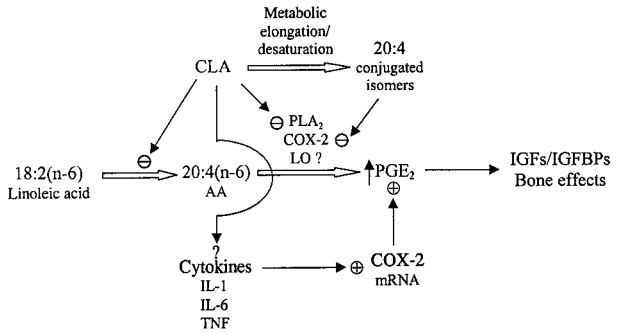


**Fig. 2.** Proposed mechanisms for the actions of CLA on $PGE_2$ production (from AA) and bone metabolism. Block arrows indicate biochemical reaction processes where CLA and linoleic acid participate. Line arrows indicate possible effects of CLA and its metabolites on $PGE_2$ metabolism and their subsequent action on bone [34]. Phospholipase $A_2$=$PLA_2$. Lipoxygenase enzyme=LO.

prostanoid biosynthesis by its isomeric analogs. Sebedio *et al.* [61] reported that CLA may be further desaturated and elongated to form conjugated C20:4 isomers which might block the access of arachidonic acid to COX. The unusual C20:4 isomers derived from CLA might also affect the activity of the COX enzymes. Further study on CLA isomers will confirm any affects of its analogs on COX activity and expression.

Dietary CLA effects on serum concentrations of IGF-I and IGF binding proteins (IGFBP) and their subsequent impact on bone modeling were examined in rats. Weanling male rats were provided AIN-93G diet containing 70 g/kg of added fat for 42 days. Treatments included 0 or 10 g/kg of CLA, and n-6 fatty acids in soybean oil (SBO) or n-3 fatty acids in menhaden oil+safflower oil (MSO) following a 2×2 factorial design [34]. Serum IGFBP was influenced by n-6 and n-3 PUFA and CLA ($p$=0.01 for 38–43 KDa bands corresponding to IGFBP-3). CLA increased IGFBP level in rats given SBO ($p$=0.05), but reduced it in those on MSO ($p$=0.01). Rats given the MSO diet had the highest serum IGFBP-3 level. This study also showed that CLA decreased liver IGF-I mRNA level in rats provided MSO supplemented with CLA ($p$=0.02) (unpublished findings). Liver IGF-I mRNA expression was up-regulated by n-3 fatty acids and down-regulated by CLA in rats. In tibia, rats given CLA had reduced mineral apposition rate (3.69 *vs.* 2.79 $\mu$m/d) and bone formation rate (0.96 *vs.* 0.65 $\mu m^3/\mu m^2$/d). Dietary lipid treatments did not affect serum intact osteocalcin or bone mineral content. These results showed that dietary PUFA type and CLA modulate growth factors that regulate bone metabolism.

In rats given CLA, bone metabolism may be altered by modulating the production of the bone resorptive cytokines, such as interleukins (IL-1 and IL-6) and tumor necrosis factor (TNF) or the lipoxygenase product leukotriene $B_4$ ($LTB_4$) (Fig. 2). IL-1 and IL-6 have long been implicated in the pathophysiology of bone diseases such as rheumatoid arthritis [62] and postmenopausal osteoporosis [63,64]. Dietary CLA was recently shown to lower basal and lipopolysaccharide (LPS) stimulated IL-6 production and basal TNF production by rat resident peritoneal macrophages [35]. Moreover, CLA may potentially reduce the release of $LTB_4$, (a lipoxygenase product of arachidonic acid), a strong bone resorption factor [65]. Assuming CLA has similar effects on these cytokines in bone, together with the fact that CLA reduced the production of $PGE_2$ in bone tissue, one could hypothesize that at a proper dietary level, the anti-inflammatory effects of CLA would be beneficial for the treatment of inflammatory bone disease.

This research is the first to show that CLA (naturally occurring in milk fat) affects bone metabolism in rats. The levels of supplemental CLA used in these studies (1% dietary CLA), though higher than that found in conventional diets without supplementation, compare favorably to the ranges used (0.5% to 1.5%) in other research that examined anti-inflammatory and anti-carcinogenic properties of CLA. Further work is needed to evaluate more typical dietary levels of CLA as research elucidates the actions of these fatty acids on bone metabolism and health.

## SUMMARY

Anti-inflammatory diets, including nutraceutical n-3 fatty acids, are associated with decreased pathogenesis of rheumatoid arthritis (secondary osteoporosis), reduced inflammatory diseases [66–68] and lowered cancer risk [69]. The common link between these diseases resides in the regulation/expression of COX-2. For example, multiple lines of evidence indicate that up-regulation of COX-2 contributes to tumorigenesis and inflammation, providing tissue levels of prostanoid precursors that influence formation of the pro-inflammatory $PGE_2$. In addition, chronic aspirin users (COX inhibitor) have reduced incidence of colorectal cancer. Both COX-1 and COX-2 inhibitors suppress experimental mouse skin carcinogenesis, and permanent overactivation of arachidonic acid metabolism appears to be a driving force for tumor development [70]. Moreover, metastasis of cancer to bone is a frequent outcome of breast (about two-thirds of patients with metastatic breast cancer have bone involvement) and prostate malignancies. The metastasis is often associated with significant morbidity (severe bone pain and pathologic fractures) due to osteolysis, and metastatic target bone is continually being remodeled under the influence of factors produced locally and systemically [71].

Interestingly, recent investigations suggest that both COX-2 induction and an increase in the supply of arachidonic acid are needed to greatly increase prostanoid production [72]. Supplying arachidonic acid appears to increase prostanoids to reduce the effects of nonsteroidal anti-inflammatory drugs, including NS-398 a specific COX-2 inhibitor. Therefore, in our view, n-3 fatty acids and CLA isomers may act as potent anticancer nutrients because they not only directly/indirectly affect the activity and expression of COX-2, but may also reduce the supply of arachidonic acid to diminish prostanoid biosynthesis.

In any case, one mode of action for CLA appears to be anti-inflammatory with respect to reducing $PGE_2$ production.

The data presented in this review describe consistent and reproducible effects of CLA isomers on decreasing *ex vivo* $PGE_2$ production in bone organ cultures [33,34] and in various cell culture systems [51]. The potent beneficial anticancer effect of CLA is likely linked, in part, to down-regulation of COX-2 activity. Future investigations on CLA should evaluate isomeric effects on COX-1 and COX-2, for which over-expression of the latter is associated with carcinogenesis and inflammation. This research would lead to 1) important discoveries for bone modeling and remodeling, 2) development of delivery systems in designed/functional foods and 3) opportunities to identify a synergism between nutraceuticals and drug therapies to reduce cancer risk and control inflammatory bone/joint disease.



## REFERENCES


1. Baron R: Anatomy and ultrastructure of bone. In Favus MJ: "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism," 2nd ed. New York: Raven Press, pp 3–9, 1993.
2. Baylink DJ, Finkelman RD, Mohan S: Growth factors to stimulate bone formation. J Bone Miner Res 8:S565–S572, 1993.
3. Mundy GR: Cytokines and growth factors in the regulation of bone remodeling. J Bone Miner Res 8:S505–S510, 1993.
4. Raisz LG: Bone cell biology: new approaches and unanswered questions. J Bone Miner Res 8:S457–S465, 1993.
5. Canalis B: Regulation of bone remodeling. In Favus MJ: "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism," 2nd ed. New York: Raven Press, pp 33–37, 1993.
6. Blair HC, Schlesinger PH, Ross FP, Teitelbaum SL: Recent advances toward understanding osteoclast physiology. Clin Orthop 294:7–22, 1993.
7. Frost HM: "Bone Remodeling and its Relationship to Metabolic Bone Disease." Springfield, IL: Thomas, pp 28–53, 1993.
8. Frost HM: "Bone Remodeling Dynamics." Springfield, IL: Thomas, 1963.
9. Kanis JA (ed): "Vol 4: Calcium Metabolism." In Parfitt AM (ed): "Progress in Basic and Clinical Pharmacology." Basel: Karger, pp 1–27, 1990.
10. Watrous DA, Andrews BS: The metabolism and immunology of bone. Semin Arthritis Rheum 19:45–65, 1989.
11. Nilsson A, Ohlsson C, Isaksson OG, Lindahl A, Isgaard J: Hormonal regulation of longitudinal bone growth. Eur J Clin Nutr 48:S150–S160, 1994.
12. Chow J, Tobias JH, Colston KW, Chambers TJ: Estrogen maintains trabecular bone volume in rats not only by suppression of bone resorption but also by stimulation of bone formation. J Clin Invest 89:74–78, 1992.
13. Raisz LO: Recent advances in bone cell biology: Interactions of vitamin D with other local and systemic factors. J Bone Miner Res 9:191–197, 1990.
14. Kream BE, Petersen DN, Raisz LG: Parathyroid hormone blocks the stimulatory effect of insulin-like growth factor-I on collagen synthesis in cultured 21-day fetal rat calvariae. Bone 11:411–415, 1990.
15. Klaushofer K, Hoffmann O, Gleispach H, Leis H-J, Czerwenka E, Koller K, Peterlik M: Bone-resorbing activity of thyroid hormones is related to prostaglandin production in cultured neonatal mouse calvaria. J Bone Miner Res 4:305–312, 1989.
16. Lukert BP, Raisz LG: Glucocorticoid-induced osteoporosis: Pathogenesis and management. Ann Intern Med 112:352–364, 1990.
17. Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, Kolakowski LF Jr, Lodish HF, Goldring SR: Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science 254: 1022–1024, 1991.
18. Igarashi K, Hirafuji M, Adachi H, Shinoda H, Mitani H: Role of endogenous $PGE_2$ in osteoblastic functions of a clonal osteoblast-like cell, MC3T3-E1. Prostaglandins Leukot Essent Fatty Acids 50:169–172, 1994.
19. Marks SC, Miller SC: Prostaglandins and the skeleton: the legacy and challenges of two decades of research. Endocrine J 1:337–344, 1993.
20. Canalis E, Centrella M, McCarthy TL: Regulation of the insulin-like growth factor-II production in bone cultures. Endocrinology 129:2457–2462, 1991.
21. Mohan S, Baylink DJ: Bone growth factors. Clin Orthop 263:30–48, 1991.
22. McCarthy TL, Centrella M, Raisz LG, Canalis E: Prostaglandin $E_2$ stimulates insulin-like growth factor I synthesis in osteoblast-enriched cultures from fetal rat bone. Endocrinology 128:2895–2900, 1991.
23. Norrdin RW, Jee WS, High WB: The role of prostaglandins in bone in vivo. Prostaglandins Leukot Essent Fatty Acids 41:139–149, 1990.
24. Parodi PW: Cows' milk fat components as possible anticarcinogenic agents. J Nutr 127, 1055–1060:1997.
25. Ip C: Review of the effects of trans fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. Am J Clin Nutr 66:1523S–1529S, 1997.
26. Ip C, Jiang C, Thompson HJ, Scimeca JA: Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis. Carcinogenesis 18:755–759, 1997.
27. Cunningham DC, Harrison LY, Schultz TD: Proliferative responses of normal human mammary and MCF-7 breast cancer cells to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture. Anticancer Res 17:197–204, 1997.
28. Visonneau S, Cesano A, Tepper SA, Scimeca JA, Santoli D, Kritchevsky D: Conjugated linoleic acid suppresses the growth of human breast adenocarcinoma cells in SCID mice. Anticancer Res 17:969–974, 1997.
29. Sehat N, Yurawecz MP, Roach JAG, Mossoba MM, Kramer JKG, Ku Y: Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33:217–221, 1998.
30. Kramer JKG, Parodi PW, Jensen RG, Mossoba MM, Yurawecz MP, Adlof RO: Rumenic acid: A proposed common name for the major conjugated linoleic acid isomer found in natural products. Lipids 33:835, 1998.

31. Ha YL, Storkson J, Pariza MW: Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res 50:1097–1101, 1990.
32. Cornell KK, Waters DJ, Coffman KT, Robinson JP, Watkins BA: Conjugated linoleic acid inhibited the in vitro proliferation of canine prostate cancer cells. FASEB 11:A579 (Abstract), 1997.
33. Li Y, Watkins BA: Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin $E_2$ biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 33:417–425, 1998.
34. Li Y, Seifert MF, Ney DM, Grahn M, Grant AL, Allen KDG, Watkins BA: Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. J Bone Miner Res 14:1153–1162, 1999.
35. Turek JJ, Li Y, Schoenlein IA, Allen KGD, Watkins BA: Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n-6:n-3 fatty acid ratio. J Nutr Biochem 9:258–266, 1998.
36. Sugano M, Tsujita A, Yamasaki M, Yamada K, Ikeda I, Kritchevsky D: Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. J Nutr Biochem 8:38–43, 1997.
37. Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K: Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids 33:521–527, 1998.
38. Wuthier RE: Involvement of cellular metabolism of calcium and phosphate in calcification of avian growth plate cartilage. J Nutr 121:301–309, 1993.
39. Gunnes M, Lehmann EH: Dietary calcium, saturated fat, fiber and vitamin C as predictors of forearm cortical and trabecular bone mineral density in healthy children and adolescents. Act Paediatr 84:388–392, 1995.
40. Watkins BA, Shen C-L, Allen KGD, Seifert MF: Dietary (n-3) and (n-6) polyunsaturates and acetylsalicylic acid alter ex vivo $PGE_2$ biosynthesis, tissue IGF-I levels, and bone morphometry in chicks. J Bone Miner Res 11:1321–1332, 1996.
41. Watkins BA, Shen C-L, McMurtry JP, Xu H, Bain SD, Allen KGD, Seifert MF: Dietary lipids modulate bone prostaglandin $E_2$ production, insulin-like growth factor-I concentration and formation rate in chicks. J Nutr 127:1084–1091, 1997.
42. Hodgson JM, Wahlqvist ML, Boxall JA, Balazs ND: Can linoleic acid contribute to coronary artery disease? Am J Clin Nutr 58: 228–234, 1993.
43. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Almazan F, Mattson FH, Khoo JC, Steinberg D, Witztum JL: Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. Am J Clin Nutr 54:701–706, 1991.
44. Watkins BA: Regulatory effects of polyunsaturates on bone modeling and cartilage function. World Rev Nutr Dietetics 83:38–51, 1998.
45. McCarthy TL, Ji C, Casinghino S, Centrella M: Alternate signaling pathways selectively regulate binding of insulin-like growth factor I and II on fetal rat bone cells. J Cell Biochem 68:446–456, 1998.
46. Xu H, Watkins BA, Adkisson HD: Dietary lipids modify the fatty acid composition of cartilage, isolated chondrocytes and matrix vesicles. Lipids 29:619–625, 1994.
47. Xu H, Watkins BA, Seifert MF: Vitamin E stimulates trabecular bone formation and alters epiphyseal cartilage morphometry. Calcif Tissue Int 57:293–300, 1995.
48. Watkins BA, Xu H, Turek JJ: Linoleate impairs collagen synthesis in primary cultures of avian chondrocytes. Proc Soc Exp Biol Med 212:153–159, 1996.
49. Adkisson HD, Risener FS, Zarrinkar PP, Walla MD, Christie WW, Wuthier RE: Unique fatty acid composition of normal cartilage: discovery of high n-9 eicosatrienoic acid and low levels of n-6 polyunsaturated fatty acids. FASEB J 5:344–353, 1991.
50. Seifert MF, Watkins BA: Role of dietary lipid and antioxidants in bone metabolism. Nutr Res 17:1209–1228, 1997.
51. Watkins BA, Seifert MF, Allen KGD: Importance of dietary fat in modulating $PGE_2$ responses and influence of vitamin E on bone morphometry. World Rev Nutr Diet 82:250–259, 1997.
52. Chen Y: "Modulatory Aspects of Polyunsaturated Fatty Acids on Epiphyseal Chondrocyte Function." [MS Thesis.] West Lafayette, IN: Purdue University, 1997.
53. Ip C, Briggs SP, Haegele AD, Thompson HJ, Storkson J, Scimeca JA: The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcinogenesis 17:1045–1050, 1996.
54. Maciel FMB, Sarrazin P, Morisset S, Lora M, Patry C, Dumais R, Debrumfernandes AJ: Induction of cyclooxygenase-2 by parathyroid hormone in human osteoblasts in culture. J Rheumatol 24: 2429–2435, 1997.
55. Suda M, Tanaka K, Yasoda A, Natsui K, Sakuma Y, Tanaka I, Ushikubi F, Narumiya S, Nakao K: Prostaglandin $E_2$ ($PGE_2$) autoamplifies its production through EP 1 subtype of PGE receptor in mouse osteoblastic MC3T3-E1 cells. Calcif Tissue Int 62:327–331, 1998.
56. Nanji AA, Zakim D, Rahemtulla A, Daly T, Miao L, Zhao SP, Khwaja S, Tahan SR, Dannenberg AJ: Dietary saturated fatty acids down-regulate cyclooxygenase-2 and tumor necrosis factor-alpha and reverse fibrosis in alcohol-induced liver disease in the rat. Hepatology 26:1538–1545, 1997.
57. Singh J, Hamid R, Reddy BS: Dietary fat and colon cancer - modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis. Cancer Res 57:3465–3470, 1997.
58. Hurley MM, Lee SK, Raisz LG, Bernecker P, Lorenzo J: Basic fibroblast growth factor induces osteoclast formation in murine bone marrow cultures. Bone 22:309–316, 1998.
59. Ono K, Akatsu T, Murakami T, Nishikawa M, Yamamoto M, Kugai N, Motoyoshi K, Nagata N: Important role of $EP_4$, a subtype of prostaglandin (PG) E receptor, in osteoclast-like cell formation from mouse bone marrow cells induced by $PGE_2$. J Endocrinol 158:R1–R5, 1998.
60. Prunzanski W, Stefanski E, Vadas P, Kennedy BP, van den Bosch H: Regulation of the cellular expression of secretory and cytosolic phospholipases $A_2$, and cyclooxygenase-2 by peptide growth factor. Biochim Biophys Acta 1403:47–56, 1998.
61. Sebedio JL, Juaneda P, Dobson G, Ramilison I, Martin JC, Chardigny JM, Christie WW: Metabolites of conjugated isomers of linoleic acid (CLA) in the rat. Biochim Biophys Acta 1345:5–10, 1997.
62. Arend WP, Dayer JM: Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 33:305–315, 1990.

63. Pacifici R, Rifas L, McCracken R, Vered I, McMurty C, Avioli LV, Peck WA: Ovarian steroid treatment blocks a postmenopausal increase in blood monocyte interleukin 1 release. Proc Natl Acad Sci USA 86:2398–2402, 1989.
64. Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, Manolagas SC: 17$\beta$-estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. J Clin Invest 89:883–891, 1992.
65. Garcia C, Boyce BF, Gilles J, Dallas M, Qiao M, Mundy GR, Bonewald LF: Leukotriene $B_4$ stimulates osteoclastic bone resorption both in vitro and in vivo. J Bone Miner Res 11:1619–1627, 1996.
66. Kremer JM: Effects of modulation of inflammatory and immune parameters in patients with rheumatic and inflammatory disease receiving dietary supplementation of n-3 and n-6 fatty acids. Lipids 31:S243–S247, 1996.
67. Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J: Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. Arthritis Rheum 37:824–829, 1994.
68. Shapiro JA, Koepsell TD, Voigt LF, Dugowson CE, Kestin M, Nelson JL: Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. Epidemiology 7:256–263, 1996.
69. Rose DP: Dietary fatty acids and prevention of hormone-responsive cancer. Proc Soc Exp Biol Med 216:224–233, 1997.
70. Marks F, Furstenberger G, Muller-Decker K: Arachidonic acid metabolism as a reporter of skin irritancy and target of cancer chemoprevention. Toxicol Lett 96–97:111–118, 1998.
71. Orr FW, Kostenuik P, Sanchez-Sweatman OH, Singh G: Mechanisms involved in the metastasis of cancer to bone. Breast Cancer Res Treat 25:151–163, 1993.
72. Hamilton LC, Mitchell JA, Tomlinson AM, Warner TD: Synergy between cyclooxygenase-2 induction and arachidonic acid supply in vivo: consequences for nonsteroidal anti-inflammatory drug efficacy. FASEB J 13:245–251, 1999.

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