FATTY ACID MODIFIERS OF BIOCHEMICAL AND MOLECULAR ACTIONS IN BONE

B.A. WATKINS

Summary

New research indicates that dietary lipids influence bone formation rates in animals and collagen synthesis in chondrocyte cultures. Feed sources of fatty acids are hypothesized to modulate the local biosynthesis of eicosanoids in bone to alter formation rates, but may also affect the production of reactive oxygen species in epiphyseal cartilage. For example, bone modeling was optimal in chicks when (n-3) fatty acids were supplied in the diet to moderate the effects of (n-6) fatty acids. Feed sources of (n-3) fatty acids elevated the concentrations of 20:5(n-3) and 22:6(n-3) in epiphyseal and articular cartilage, and in cortical and trabecular bone in chicks. Moreover, (n-3) fatty acids reduced the concentration of 20:4(n-6) in bone polar lipids, decreased ex vivo prostaglandin E₂ (PGE₂) production in bone organ culture, increased bone formation rate, and improved mechanical properties of bone. These observations are believed to be the result of long-chain (n-3) fatty acids affecting osteoblastic activity by modifying autocrine and paracrine signals that govern bone modeling. Since PGE₂ exhibits biphasic effects on bone formation, stimulating bone formation at a low concentration but inhibiting it at higher concentrations, (n-3) fatty acids may directly enhance osteoblastic bone formation. Another potential effect of (n-3) fatty acids may be up-regulation of insulin-like growth factor-1 (IGF-1) anabolic action on bone. The recent discovery of the Cbfa1 gene, which controls differentiation of osteoblasts in animals, provides an opportunity to explore nutrient gene regulation of bone formation. The Cbfa1 gene controls other genes that influence osteoblastic bone formation. A lack of its expression results in boneless mice which have only cartilage (Ducy et al., 1997). The relationship between nutrients and gene expression is relatively new, and recent research demonstrated that dietary fatty acids regulate hepatic gene transcription. This paper presents research that describes how fats and antioxidants support bone formation and benefit cartilage function to optimize bone modeling in poultry.

I. BONE CELLS AND BONE METABOLISM

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 vitamind₃] and autocrine and paracrine factors, including prostaglandins, cytokines, and growth factors orchestrate the cellular activities of bone modeling to increase the length, diameter, and shape of long bones in animals. Bone growth includes the activities of bone matrix formation, matrix mineralisation, and bone resorption. Bone matrix is produced and mineralised through the activity of osteoblasts while bone matrix resorption is accomplished by specialized multinucleated cells called osteoclasts. The combined and cooperative activities of osteoblasts and osteoclasts result in a bone architecture that provides mechanical support and maintains normal serum concentrations of calcium and phosphorus. The effects of feed fats on altering the amount of local factors produced in bone will be the focus of this paper.

Lipid Chemistry and Molecular Biology Laboratory, Department of Food Science, Purdue University, West Lafayette, IN, 47907-1160, USA.
II. BONE GROWTH

Bone growth and modeling are regulated by complex interactions between an individual's genetic potential, environmental influences, and nutrition. These interactions produce a bone architecture that balances functionally appropriate morphology with the skeleton's role in calcium and phosphorus homeostasis. Long bones of poultry increase in length and diameter by a process called modeling. Bone modeling represents an adaptive process of generalized and continuous growth and reshaping of bone governed by the activities of osteoblasts and osteoclasts until adult bone structure is attained in poultry. This growth requires that bone cells function normally. Bone modeling is distinct from bone remodeling which describes the local, coupled process of bone resorption and formation that maintains skeletal mass and morphology in the mature hen.

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 (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. Osteoblasts deposit organic matrix within their embryonic connective tissue membrane which becomes mineralized. Long bones are formed by endochondral ossification, a process where embryonic mesenchymal cells differentiate into chondroblasts which secrete hyaline cartilage matrix and produce a cartilage model of the future bone. Diaphyseal and, later, epiphyseal centers of ossification develop following local cartilage mineralisation and invasion by the vasculature. Cartilage matrix is removed and replaced with bone by newly arrived osteogenic cells. The location of a 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 is balanced with removal of mineralized trabeculae from the metaphyseal side of the growth plate through osteoclastic activity. 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.

III. REGULATION OF BONE METABOLISM

Bone formation and bone resorption are regulated by systemic hormones and factors produced locally primarily by osteoblasts (Watkins, 1992). Systemic hormones involved in stimulating bone formation include, insulin, growth hormone, and estrogen; while those involved in stimulating bone resorption include, 1,25-(OH)$_2$ vitamin D$_3$, PTH, and thyroid hormone. In addition, calcitonin and glucocorticoids inhibit bone resorption.

IGF-1, also called somatomedins, are described as paracrine or autocrine regulatory polypeptides of cells. These compounds stimulate growth and synthesis of DNA, RNA, and proteins in cells. IGF are mitogenic and stimulate differentiation in a variety of cell types. Pituitary growth hormone (GH) controls tissue biosynthesis and secretion of IGF-1 (or somatomedin C) postnatally and it is through IGF-1 that the tissue effects of GH are mediated.
Serum concentration of IGF-1 is maintained by liver synthesis under the influence of GH. Much of the circulating IGF is bound to plasma IGF binding proteins (IGFBP). The amount of IGF-1 and IGF-2 produced by bone cells is species dependent. In the human, neonatal mouse, and chicken more IGF-2 than IGF-1 is produced in the skeletal tissues (Baustista et al., 1991). While IGF-2 is generally more abundant than IGF-1, IGF-1 appears to be under greater regulatory control in bone (Canalis et al., 1991). For example, prostaglandin E₂ (0.01–1 μM) elevated IGF-1 mRNA and polypeptide levels by 1.9- to 4.7-fold; however, prostaglandin E₂ did not increase IGF-2 mRNA or polypeptide levels in bone organ cultures (McCarthy et al., 1991).

In addition to the cytokines and growth factors which act as local modifiers of bone metabolism, certain eicosanoids [e.g., prostaglandins (PG), leukotrienes (LT)] also exert stimulatory effects on bone formation and resorption (Table 1). In 1970, Klein and Raisz (1970) reported that PGE₁, PGE₂, PGA₁, and PGF₁₀ increased the release of ⁴⁵Ca into the media from cultured fetal rat bone. Since then, numerous studies have demonstrated that PGEs stimulate bone formation as well as bone resorption (Raisz, 1993; Marks and Miller, 1993). Raisz (1993) reported that infusion of PGE₂ at a high concentration depressed osteogenesis in fetal rat calvariae. PGE₂ stimulates bone formation at low concentrations but it may be inhibitory at high concentrations.

Similar to the PG, the LT also play an important role in bone metabolism. Ren and Dziak (1991) demonstrated that LTB₄ inhibited cell proliferation in cultured osteoblasts isolated from rat calvaria in a dose-dependent manner, but LTB₄ may interact with PG to regulate osteoblast activity. Other reports indicate that LTC₄, LTD₄, and 5-HETE stimulated isolated avian osteoclasts to resorb bone.

IV. DIETARY LIPIDS MODIFY THE FATTY ACID COMPOSITION OF BONE

Although the importance of lipids in cartilage mineralisation and bone biology has been well documented, research describing the relationships between dietary lipids and chondrocyte function and prostanoid effects on bone formation has, until recently, received little attention (Watkins and Seifert, 1996). Analysis of epiphyseal cartilage in animals revealed a low concentration of (n-6) fatty acids and 3-4% Mead acid [20:3(n-9)] (Adkisson et al., 1991). [Mead acid accumulates in animal tissues during a deficiency of the essential fatty acid linoleic acid.] Mead acid was not reduced in cartilage of chicks given a rich dietary source of 18:2(n-6); however, consumption of (n-3) fatty acids [20:5(n-3) and 22:6(n-3)] elevated their concentration in cartilage (Xu et al., 1994). These findings indicate that epiphyseal cartilage, which is responsible for longitudinal bone growth, may selectively incorporate certain dietary fatty acids.

Table 1. Reported responses of autocrine and paracrine factors in bone

<table>
<thead>
<tr>
<th>Responses observed in bone</th>
<th>Cytokine, eicosanoid, or peptide growth factor²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone formation or matrix production</td>
<td>FGF, IGF, PGE, TGF-β</td>
</tr>
<tr>
<td>Bone resorption</td>
<td>EGF, IL, LT, PDGF, TGF-α, TNF-α</td>
</tr>
<tr>
<td>Collagen synthesis</td>
<td>FGF, IGF, TGF-β</td>
</tr>
</tbody>
</table>

¹Adapted from Watkins (2). ²Epidermal growth factor = EGF; Fibroblast growth factor = FGF; Interleukin = IL; Insulin-like growth factor = IGF; Leukotriene = LT; Platelet-derived growth factor = PDGF; Prostaglandin E = PGE₂; Transforming growth factor = TGF-α, TGF-β; Tumor necrosis factor = TNF-α.
Recent investigations suggest that vitamin E benefits bone growth and cartilage activity. Chicks given supplemental vitamin E demonstrated higher bone formation rates (Xu et al., 1995). In cartilage, the ability to protect against lipid peroxidation may be restricted to nonmineralized regions of cartilage. Research indicated that the mineralized area of growth plate cartilage has limited enzymatic capacity for handling oxidized lipid species because superoxide dismutase and catalase activities are low in this region. The enrichment of chicken epiphyseal chondrocytes with 18:2(n-6) resulted in cellular injury [elevated lactate dehydrogenase (LDH) activity] and depressed collagen synthesis when compared to cells supplemented with oleic acid and no fatty acids (Watkins et al., 1996). Consistent with the effect of linoleic acid on chondrocyte injury (elevated LDH activity in culture media), enrichment with 18:2(n-6) lowered chondrocyte collagen synthesis; however, vitamin E restored collagen synthesis in these cells. Accompanied with the reduction in collagen synthesis in primary cultures of epiphyseal chondrocytes is an elevated production of PGE₂ (Watkins and Chen, 1997). The decrease in collagen synthesis observed with (n-6) fatty acid enrichment appears to be related to membrane damage and impaired cell function, for which vitamin E is protective. Since the onset of peroxidative reactions within biological membranes can impair cell behavior and function, vitamin E and antioxidant systems designed to protect chondrocytes may be minimal in epiphyseal cartilage. Collectively, these data suggest that diets marginal in vitamin E or which lead to oxidative stress (linoleic acid) and tissue depletion of vitamin E, impair normal bone and cartilage function.

Studies with chicks demonstrated that dietary lipids modify the fatty acid composition of cortical and trabecular bone. Chicks given diets containing trans-18:1 or (n-3) fatty acids had increased concentrations of these fatty acids in bone. The conjugated linoleic acid (CLA) isomers in anhydrous butter oil were also found in bone tissues of animals given CLA (Li and Watkins, 1998). Watkins et al. (1996, 1997) reported that chicks given a blend of menhaden oil + safflower oil in a semi-purified diet had a lower concentration of 20:4(n-6) but higher concentrations of 20:5(n-3) and 22:6(n-3) in cortical bone polar lipids compared to those given soybean oil. As the concentration of 20:4(n-6) decreased in tibial bone of chicks given 20- and 22-carbon (n-3) fatty acids so did the ex vivo PGE₂ production in bone organ culture (Watkins et al., 1996, 1997). Since diets that moderate ex vivo PGE₂ production in bone organ culture were associated with higher rates of bone formation in vivo, it is presumed that dietary lipids [(n-3) fatty acids and CLA] impact bone formation and resorption activities by modulating PGE₂ biosynthesis.

V. DIETARY FATTY ACIDS ALTER BONE FORMATION

Determining the consequence of altering the fatty acid composition of cartilage and bone with dietary lipids has been one aim of our research. Our investigations indicate that dietary lipids influence bone formation and chondrocyte cell function. For example, kinetic analyses of bone modeling revealed that total fractional labeled trabecular surfaces and bone formation rate (BFR) were significantly greater in chicks given menhaden oil + safflower oil compared to those given soybean oil (Watkins et al., 1996). A rather intriguing observation was that the increased BFR in chicks given 20- and 22-carbon (n-3) fatty acids was associated with a 3.5-fold decrease in ex vivo PGE₂ production in tibia. Under this dietary condition 20:5(n-3) predominates over arachidonic acid as an eicosanoid precursor since its concentration was 10-fold higher, while arachidonic acid concentration was about 50% lower in tibia. Thus, principle metabolites from 20:5(n-3) might include PGE₃ or leukotrienes (LTB₃, LTC₃, LTD₃, LTE₃). Providing aspirin in the diet of these animals abolished ex vivo PGE₂ (20 to 36-fold decrease) production in bone but BFR was sustained. The effects of dietary fatty acids and related factors
on the osteoblast and osteoclast are summarized in Figure 1.

Fig. 1. Observed and potential effects of dietary fatty acids and related compounds on osteoblastic and osteoclastic activity in bone. Excessive biosynthesis of PGE₂ may depress bone formation and lead to increased bone resorption. Altering the production of eicosanoids (PGE and LTB₅) appears to optimize bone formation by osteoblasts and moderate bone resorption by osteoclasts. Vitamin E may benefit bone formation and reduce excessive bone resorption by decreasing free radicals.

PGE₂ exhibits biphasic effects on bone formation, stimulating bone formation at a low concentration but inhibiting it at higher concentrations, and excess production of PGE₂ is perhaps associated with bone pathology. The higher amount of bone PGE₂ in chicks given soybean oil could have stimulated an increase in bone resorptive activity that reduced bone volume and trabecular number. It appears that dietary 20- and 22-carbon (n-3) fatty acids aid in moderating PGE₂ production in bone to optimize bone formation and perhaps prevent excessive bone resorption.

VI. CONCLUSIONS

Recent data demonstrating dietary lipid effects on bone metabolism indicate that dietary 20- and 22-carbon (n-3) fatty acids reduce skeletal production of PGE₂ to enhance bone formation and optimize bone modeling. Improved bone modeling may reduce the incidence of abnormal bone modeling in rapidly growing chickens. Furthermore, antioxidant nutrients may enhance bone formation and reduce the production of free radicals which contribute to bone resorption (Watkins et al. 1997b). Because fat constitutes a significant amount of the energy in poultry rations, the type of fat consumed can significantly influence the metabolic and physiological processes controlling bone modeling in animals. Likewise, the levels of antioxidant nutrients and flavonoids may contribute to better bone growth by reducing the formation of free radicals and lipid peroxides.
The production of PGE₂ in bone was significantly decreased in chicks consuming menhaden oil compared to the amount in those given soybean oil. Decreasing bone resorption and stimulating bone formation with dietary (n-3) fatty acids may afford a means to maximize bone mineral accretion in growing animals and minimize mineral mass loss in the laying hen. Future research on fats and antioxidant compounds in bone biology will benefit poultry and other livestock. This work can now be directed at nutrient gene regulation of bone formation because of the recent discovery of the Cbfal "master gene" which influences osteoblastic activity. Research on the Cbfal gene might provide some insight on how (n-3) fatty acids increase bone formation in the chicken.

ACKNOWLEDGMENTS

This research was supported by USDA/NRI grant no. 96-35200-3137.

REFERENCES