

Bone: More Than a Stick

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ABSTRACT: Despite the importance of the skeleton for animal welfare, research into the skeletal biology of farmed species is relatively neglected. This review describes basic concepts relating to current

knowledge of the control processes responsible for bone growth and its subsequent remodeling from a physiological, cell biological, and mechanical function perspective.

Key Words: Bone Cells, Hormones, Growth Factors, Biomechanics

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Introduction

Until recently, the importance of the skeleton in terms of animal production has been sadly neglected. This is primarily because it has little economic value so that the search for efficiency in agriculture was based on better feed conversion and concentrated either on muscle development or on reproduction. The realization, initially in poultry but more recently in other species, that rapid growth rates and over-emphasis on muscle mass was in fact deleterious to the skeleton and other body systems has highlighted the need for better understanding of the control and possible manipulation of skeletal growth and development. Unfortunately, research on the skeleton has not always attracted the interest of scientists because it has been considered to be relatively inert. The aim of this article, therefore, is to provide a brief and basic description of what is currently known about the control of skeletal development and its turnover with the goal of making research on the skeleton a matter of greater interest to animal scientists in general.

What is the Role of the Skeleton?

The skeleton has three main roles; the difficulty is deciding which one is preeminent. First, it protects internal organs such as the brain and spinal cord, heart, and lungs. Second, it acts as an attachment site for muscles and as levers for locomotion. Consequent

upon this role is the concept of skeletal adaptation to the loads applied upon it (Rubin and Lanyon 1987). Third, it acts as a calcium reservoir in times of chronic, rather than acute, calcium stress. Although generalizations have been made regarding the skeletal site for these last two roles, with cortical bone being primarily involved in support and cancellous bone as the site of metabolic activity, factors such as parathyroid hormone (PTH), which primarily controls calcium homeostasis, also seem to be important in skeletal integrity so that it is considered as a possible treatment for osteoporosis (Reeve, 1996). Birds have developed a specific type of bone, medullary bone, which can be quickly formed and resorbed in response to the extreme demand for calcium during the egg-laying cycle.

Types of Bone

Bone consists of a mineral phase enmeshed within organic fibers. Although it is generally considered that the mineral resists compression and the collagen fibers withstand torsion and tension, the orientation of these fibers seems to be load related (Riggs et al., 1993). Cortical (compact or lamellar) bone consists of concentric layers of matrix surrounding longitudinal vessels within the structure (Haversian systems). Between these lies mainly unremodeled interstitial bone. Cortical bone is found in the shafts of the appendicular skeleton; cancellous (trabecular) bone is

found predominantly in the axial skeleton and at the epiphyses of the long bones. This type of bone consists of a framework of bony struts which, although weaker than cortical bone, provides a metabolically active lightweight support. The development of medullary bone during the egg-laying cycle is unique to birds (Miller, 1992). This type of bone enables the animal to store a large amount of calcium that is rapidly available to meet the demands of egg-shell formation and is possibly the only true type of metabolic bone in the animal kingdom.

Longitudinal Bone Growth

Longitudinal bone growth results from the proliferative and metabolic activities of chondrocytes in the epiphyseal plate. The growth plate is divided into several distinct zones, perpendicular to the main axis of growth; each zone consists of chondrocytes in different phases of the chondrocyte lineage. These zones are described as layers of resting (or stem cell), proliferating, and hypertrophying chondrocytes and can be characterized by the specific shape, size, and metabolic activity of the cells. Cells from the resting zone are recruited into the proliferative zone; after a number of divisions, they stop dividing and enter a differentiated zone in which they hypertrophy and produce an abundant extracellular matrix which becomes mineralized. Following this there is now evidence that chondrocytes die by apoptosis. Bone elongation therefore results from both proliferation and hypertrophy: proliferation increases the number of chondrocytes, while hypertrophy leads to displacement of cartilage tissue away from the bone centre. Thus, the bone growth rate equals the rate of new cell production per column multiplied by the mean height of hypertrophied chondrocytes (Sissons, 1955; Hunziker and Schenk, 1989). The easy identification of cells at each point in this lineage cascade makes the study of chondrocyte cell biology *in vivo* much easier than, say, that for osteoblasts or osteoclasts.

The regulation of longitudinal growth is still not fully elucidated but it is clear that a multiplicity of both local and systemic factors are involved at each of the above stages. Growth hormone plays a major role, particularly in the commitment of the early resting cells to a proliferative phenotype (Isaksson et al., 1982). *In vitro*, prechondrocytes respond to GH, whereas IGF-I acts on the more committed progenitor cells (Lindahl et al., 1987). *In vivo* the highest concentration of GH receptors are found on the stem

cells, while those for IGF-I are predominantly present on the proliferative cells. Thus, the *in vivo* action of GH seems to be to promote the commitment of the early cells to a proliferative phenotype without necessarily affecting the rate of chondrocyte proliferation (Gevers et al., 1996). While IGF-I increases the amount of chondrocyte proliferation, it is clear from the studies on achondroplasia that fibroblast growth factor (FGF) is also important. The FGF appears to act synergistically with transforming growth factor- β (TGF β) to stimulate chondrocyte proliferation (Crabb et al., 1990). Other factors reported to be involved at this stage include the proto-oncogene *c-myc*, but this factor may also be involved in the process of switching between proliferation and differentiation (Farquharson et al., 1992; Loveridge et al., 1993).

Control of chondrocyte differentiation is also a complex issue, with traditional factors such as 1,25 dihydroxyvitamin D₃, (1,25(OH)₂D₃) being important (Farquharson et al., 1996). More recently, studies on the physiological role of parathyroid hormone related peptide (PTHrP) first identified in hypercalcaemia of malignancy (Moseley et al., 1987), have demonstrated its importance in the control of chondrocyte differentiation (Lee et al., 1996).

Mineralization

During longitudinal growth, mineralization is restricted to the extracellular matrix surrounding the hypertrophic chondrocytes (Ali, 1992). This matrix is extensively modified from that of other growth plate chondrocytes, but the exact role of individual matrix components during mineralization is contentious. How osteoid mineralizes during bone remodeling is still a matter of debate, but it seems to involve at least some of the same mechanisms as in the growth plate with the initial formation of primary nucleation sites and subsequent formation of hydroxy-apatite. In both the growth plate and osteoid, mineralization is associated with alkaline phosphatase activity (Robinson, 1923; Register and Wurthier, 1984; Bradbeer et al., 1992, 1994) but the exact role of this enzyme is unclear. In the growth plate, Type X collagen has been implicated in the initiation of matrix calcification but this is not the case during bone remodeling. Many other matrix proteins are involved in mineralization including chondrocalcin, proteoglycans, osteonectin, and osteocalcin (Termine, 1993). Changes in the degree of sulphation of proteoglycans have also been implicated (Farquharson et al., 1994).

The elasticity of bone and thus its resistance to fracture is related to its degree of mineralization, because an increase in stiffness and brittleness of bone tissue follows the replacement of its water content by mineral. The mineral content in any microvolume of bone normally increases asymptotically with its age until it is removed by osteoclasts. Thus, there is a net increase in the proportion of bone microvolumes which have a higher density with age, reflecting reduced turnover and replacement (Jowsey, 1960; Currey, 1979; Reid and Boyde, 1987; Boyde et al., 1995; Currey et al., 1995).

The relationship between mineral content and the strength of bone has been studied extensively (Vose and Kubala, 1959; Currey, 1969). Increasing mineralization density increases the ability of bone to absorb impact energy, although this relationship is not linear. However, above an ash content of 60% by weight, femoral cortical bone becomes increasingly liable to fracture, because microfractures can more readily propagate through bone that is highly mineralized (Zioupou and Currey, 1994; Currey et al., 1995). The optimal mineralization density value for bone strength has not yet been determined, although recent studies have suggested that it can be modified by estrogen status (Boyde et al., 1998).

Bone Remodeling

Apart from its role in the regulation of calcium homeostasis, bone remodeling is designed to maintain a mechanically competent skeleton (Wolff, 1892) and remove areas of microdamage (Burr et al., 1997). It achieves this by removing a quantum of bone and replacing it with new bone (Parfitt, 1996). During growth this process results in bone expansion, with bone formation exceeding bone resorption. Following growth, bone volume remains static with resorption and formation being in balance. In later life however, resorption exceeds formation, leading to a slow decline in bone mass.

Bone remodeling involves a localized cycle of osteoclastic recruitment and activation followed by the subsequent initiation of osteoblast formation and the subsequent repair of the resorption site. Within cortical bone (currently neglected) this process is achieved through osteonal remodeling, where a cutting cone of osteoclasts advances through the bone and is followed by osteoblasts making new bone (Parfitt, 1996). In cancellous bone a similar process occurs and

has been described as hemi-osteonal remodeling, whereby a trench is cut through the surface of individual trabeculae and filled in by osteoblast activity. Thus bone turnover, provided it does not result in a remodeling deficit, maintains the ability of the skeleton to withstand the physiological and mechanical demands placed upon it.

Osteoclasts

Osteoclasts are large multinucleate cells that can penetrate 50 to 70 μm into compact bone and resorb a volume of bone equivalent to that formed by 100 to 1,000 osteoblasts. Failure to produce osteoclasts or deficiencies in their action results in diseases such as osteopetrosis. Osteoclasts possess numerous mitochondria and an extensive Golgi system but have a sparse endoplasmic reticulum and few ribosomes. Contact with bone is through a "ruffled border" where resorption occurs and a peripheral "clear zone" which acts as a seal. This is to maintain an optimal acidic environment for the lysosomal enzymes involved in the degradation of both the mineral and organic phases of bone. This acidic environment is generated through the action of carbonic anhydrase Type II and pumped across the ruffled border by a proton pump (Baron 1995). Contact with the bone surface is possibly through the vitronectin receptor of integrin system (Helfrich et al., 1996). Osteoclasts are formed from the hematopoietic mononuclear cells of the bone marrow, although the exact nature of the precursor cell is still a matter of debate. The most likely stem cell is considered to be part of the granulocyte-macrophage series which then undergoes a specific differentiation pathway under the control of factors such as colony-stimulating factor-1 (Sarma and Flanagan 1996).

The regulation of osteoclast activity is complex, with a variety of factors (including systemic hormones such as PTH, $1,25(\text{OH})_2\text{D}_3$ and calcitonin as well as numerous local factors) being considered to regulate osteoclast activity (Mundy, 1993). A number of these factors act through the generation of secondary signals by osteoblasts, a mechanism which is considered to "couple" bone resorption to bone formation. A major unsolved problem in osteoclast biology is the mechanism whereby bone resorption is terminated. Possible factors include activation of matrix-derived $\text{TGF}\beta$ (Pfeilschifter et al., 1988), the presence of a calcium sensing mechanism (Kameda et al., 1988) and, finally, the control of osteoclast apoptosis (Hughes et al., 1996).

Osteoblasts

Osteoblasts are a single layer of mononuclear cells found on bone surfaces. They exist in two major forms: either as columnar matrix-producing cells or as flattened quiescent cells (sometimes called lining cells). Factors such as PTH have been suggested to act as a switch between these phenotypes (Dobnig and Turner, 1995). Although rates vary, a typical osteoblast deposits a volume of matrix equivalent to its own size every day. This matrix, termed osteoid, consists primarily of Type I collagen, but a number of non-collagenous proteins such as sialoprotein, osteocalcin, and osteonectin are also present (Termine, 1993). High concentrations of growth factors such as TGF β and IGF are also secreted into the matrix. The osteoid is then mineralized but a thin layer of unmineralized matrix covers all bone surfaces.

Osteoblasts are members of a family of fibroblast-like cells which include chondrocytes, adipocytes, and myoblasts (Freidenstein et al., 1987; Owen, 1988). The earliest identified precursor is the inducible osteogenic precursor cell which only forms bone if artificially induced (Urist et al., 1983), but once committed to the osteogenic lineage can form bone, fat cartilage, and fibrous tissues spontaneously. Maturation to pre-osteoblasts results in the expression of early markers of osteoblast differentiation such as Type I collagen, alkaline phosphatase, and osteonectin. Terminal differentiation results in the expression of late markers such as osteopontin and osteocalcin.

The control of the process of osteoblast differentiation is poorly understood, with a multiplicity of factors thought to be involved (Reddi, 1997). However, the transcription factor Cbfa-1 is thought to be important in osteoblast development (Rodan and Harada 1997). The Cbfa-1 has been identified as a "master gene" for maintaining the promoters of synthesis of the various bone matrix proteins (including alkaline phosphatase, osteocalcin, and Type 1 collagen). Mice lacking Cbfa-1 have no osteoblasts.

Although originally defined as fully differentiated cells, osteoblasts are now considered to have flexible phenotypes and variable functions. Thus, they may either form bone, regulate osteoclastic bone resorption, or become quiescent (Rodan and Martin, 1981; Chambers 1985). Whether each osteoblast can perform all these functions or whether specific subtypes are required for individual functions still has not been established. For instance, PTH receptors are more abundant on cells that are not adjacent to the osteoid surface, indicating that such cells, rather than mature osteoblasts, are involved in transducing the signals

that affect bone metabolism (Rouleau et al., 1988; Fermor and Skerry, 1995).

Loading

Adaptation of bone in response to load has been clearly elucidated by the studies of Rubin, Lanyon, and others (Rubin and Lanyon, 1987). Although this is not the case for all bones, in a cantilevered beam-like bone such as the sheep or deer calcaneus, where the loading pattern is relatively simple with one cortex in compression and the other in tension (Lanyon, 1974), the compression cortex is thicker and has an increased osteon density but has smaller osteons and less turnover, while the cortex in tension is thinner and has a higher turnover with larger, less numerous osteons (Skedros et al., 1994a). There are also differences in the degree of skeletal mineralization, with regions of bone that are in tension having a lower mineral density than those in compression (Skedros et al., 1994b).

Cortical porosity has been investigated in relation to the principal loading mode, compression or tension, experienced by the bone of interest. Studies on the artiodactyl calcaneus (Skedros et al., 1994a) have demonstrated marked differences in the porosity associated with these loading environments. The cortex in tension had a lower porosity than that in compression. The femoral neck has similar strain modes across the bone, with the superior cortex predominantly in tension and the inferior cortex in compression (Lotz et al., 1995), and similar changes in cortical porosity (Bell et al., 1999). Cortical bone remodeling varies in response to the loading environment so that analyzing small samples of bone may not present the true picture of the overall level of remodeling (Rubin et al., 1996; Iwaniec and Crenshaw, 1998). Furthermore, differing loading environments also affect collagen orientation: bone under compression has oblique/transverse collagen while that under tension has longitudinal collagen (Riggs et al., 1993).

Osteocytes

Osteocytes are ex-osteoblasts that become entrapped in bone matrix during bone formation. They inhabit the lacunar-canalicular system and communicate with their fellows and with the bone surface lining cells, in part, via gap junctions. Osteocytes in the territory of a Haversian capillary die if their blood

supply is interrupted (Jee, 1968). There is increasing evidence that the osteocyte acts as a mechano-receptor (Skerry et al., 1989; Lanyon, 1996) and that they almost certainly sense rates of change of mechanical deformation (strain). A number of paracrine signals are stimulated in osteocytes following changes in skeletal loading, including PGI₂ and PGE₂, nitric oxide, and IGF. More recently, the finding that expression of the glutamate transporter is increased following loading (Mason et al., 1997) suggests that excitatory amino acids may play a role in the transduction of the loading signal.

Recent studies have raised the intriguing possibility that osteocyte apoptosis may be part of the mechanism whereby osteoclasts are targeted to sites of bone resorption (Noble et al., 1997b), as it is elevated in bone that is being remodeled. Estrogen suppression, a known stimulant of bone resorption, increases osteocyte apoptosis (Tomkinson et al., 1997), while changes in bone loading are also associated with osteocyte apoptosis (Noble et al., 1997a).

Implications

Interactions among cell types and local and systemic factors controlling bone growth and turnover are complex, but research on the bone biology of farmed species has lagged behind other areas. This has led to an increasing incidence of skeletal problems as the demand for increased weight has outstripped the capacity of the skeleton to support the animal. Because bone often meets the calcium requirements for reproduction (e.g., eggshell formation, fetal mineralization, and milk production), earlier inadequacies can be exacerbated. Although much is known about the skeleton, bone research will continue to play a vital role in agriculture. Indeed, a lack of bone research will result in increasing losses, both in terms of production and the perception that rapid growth of farmed animals compromises their welfare. Bone research is not often considered to be on the cutting edge of scientific investigation. However, it provides an important challenge for scientists from a number of disciplines, including cell and molecular biology, physiology, nutrition, genetics, physics, and engineering.

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