Evolutionary Physiology of Bone: Bone Metabolism in Changing Environments

Bone evolved to serve many mechanical and physiological functions. Osteocytes and bone remodeling first appeared in the dermal skeleton of fish, and subsequently adapted to various challenges in terrestrial animals occupying diverse environments. This review discusses the physiology of bone and its role in mechanical and calcium homeostases from an evolutionary perspective. We review how bone physiology responds to changing environments and the adaptations to unique and extreme physiological conditions.

Bone is a very dynamic and metabolically active organ, which is composed primarily of calcium-phosphate mineral and type I collagen. Bone remodeling (the coupled actions of osteoclasts and osteoblasts) contributes to organismal mineral homeostasis and mechanical homeostasis of the skeleton. Osteoclasts produce hydrogen ions to dissolve hydroxypatite mineral crystals and hydrolytic enzymes to digest the organic matrix of bone. Osteoblasts deposit the organic matrix (i.e., osteoid), which is primarily type I collagen, and regulate the mineralization process. Some osteoblasts terminally differentiate into osteocytes, which become embedded in cavities (i.e., lacunae) in the mineralized matrix. The bone remodeling process occurs on the surfaces of trabecular struts and within cortical bone to form microstructural units known as osteons (FIGURE 1). Osteocytes have long cytoplasmic processes that extend through small channels (i.e., canaliculi) in the mineralized matrix of cortical and trabecular bone. Through the extensive lacunocanalicular network, osteocytes may transmit information (e.g., via gap-junctional communication) regarding the mechanical environment (e.g., mechanical strain), the presence of microscopic mechanical fatigue damage, and the extracellular fluid calcium concentration (15, 29). Furthermore, as the communication network extends to blood vessels, it is possible that the status of blood calcium concentration is communicated to osteocytes and that they might regulate (increase or decrease) blood calcium concentration by directing the activity of osteoclasts and osteoblasts, or directly via osteocytic osteolysis (5). Bone modeling refers to the independent actions of osteoclasts and osteoblasts to remove and produce bone matrix, respectively, during ontogeny and phenotypic plasticity in response to the mechanical environment (76).

In this review, we refer to the physiology of bone as the modeling and remodeling processes (i.e., the actions of osteocytes, osteoclasts, and osteoblasts) in regulating calcium homeostasis, fatigue damage homeostasis, and phenotypic plasticity in response to the mechanical environment. We refer to the evolutionary physiology of bone as the evolution of those processes. Bone also plays important physiological roles in reproduction and fat and energy metabolism (59), but those aspects will not be considered here. Instead, this review focuses on the evolutionary and comparative physiology of bone as it pertains to calcium and mechanical homeostases. The evolution of mineralized skeletal tissues, including histological and morphological features, has been reviewed extensively by Donoghue et al. for example (36).

Calcium homeostasis is important for all life on Earth. The regulation of cytosolic calcium concentration, by calcium channels and pumps, is evolutionarily conserved and one of the most primitive attributes of all cells (23, 101). Calcium regulation is essential to cell survival (23), and calcium is a universal regulator of numerous cellular processes such as gene expression, cell cycle, reproduction, and apoptosis (8, 10, 18). Cytosolic calcium concentrations are 10,000 to 20,000 times lower than in the extracellular milieu (FIGURE 2; Refs. 23, 56), a necessity considering high intracellular calcium results in damage to organelles, protein denaturation, DNA condensation, and cell death (17). Additionally, large calcium gradients provide cells the ability to regulate numerous cellular processes, in response to biochemical and mechanical stimuli, by varying properties of the cytosolic calcium oscillation (e.g., oscillation magnitude, duration, and frequency) (FIGURE 3) (8, 9, 17, 33, 46, 91). Thus calcium provides organisms with the potential for incredible biodiversity (17, 91).

In addition to serving as an ubiquitous signaling molecule, calcium provides vertebrates the ability to form mineralized bone tissues, which serve multiple mechanical functions such as protection, support, reproduction, and locomotion. Calcium-phosphate,
in the form of impure hydroxyapatite, bestows the stiffness and strength bones need to perform their mechanical functions (28, 69). Before the appearance of bone, calcium carbonate had been used for millions of years to form exoskeletons in marine animals (113). However, calcium phosphate is more insoluble and easier to precipitate than calcium carbonate-containing hard tissues of other organisms (69). This may have been a driving factor in the selection of hydroxyapatite for building bone tissue, especially where intense bouts of muscular contraction produced an acidic environment due to lactic acid accumulation. Additionally, switching to calcium phosphate provided a mechanism for storing phosphate in the body, which may have improved energy metabolism by increasing the ability to generate ATP (113).

Our understanding of the evolution of the physiology of bone is limited primarily to the evidence in the fossil record and information gained from extant correlates through phenotypic, genetic, and developmental comparisons (113). The fossil record, however, is typically devoid of soft structures, such as cells, tendons, and cartilage. Thus bone cells are not preserved in fossilized specimens, but their existence is inferred from the presence of microstructural features in bone tissues (51). Cells such as osteocytes, osteoclasts, and osteoblasts leave behind lacunae, canals, lamellae, and osteons (in extinct and extant species) within bone, providing evidence of their presence (50, 51). For example, bone resorption processes are clearly evident as eroded surface in early Heterostraci (jawless fish with acellular dermal bone) as an indication of early bone physiology (51).

When and how did calcium storage in bone become important for organismal calcium homeostasis? Did bone first appear to serve a mechanical or ionic regulatory function? Here, we discuss various hypotheses regarding the evolutionary physiology of bone, the implications of bone as a mineral reservoir, and the comparative physiology of various calcium and mechanical challenges found in living animals to help elucidate these questions and the physiological diversity of the skeleton. Ultimately, it is difficult to choose either the mechanical or the physiological hypothesis to explain the origin of bone and bone physiology since they are difficult to test in extinct and extant species (35). Notwithstanding, extant animals have clearly evolved numerous unique bone physiological processes to thrive

**FIGURE 1. The bone remodeling process**
A: bone remodeling occurs on the surfaces of trabecular bone found in epiphyses and metaphyses of long bones, as shown in the proximal femur, and within cortical bone (red box). B: a cross section through the diaphysis shows the secondary osteonal structure of cortical bone: completed secondary osteon (orange arrow), large remodeling cavity (green arrow), and partially refilled remodeling cavity (red arrow). C: the periphery of secondary osteons are defined by the cement line (arrows). D: extensive canalicicular network between osteocyte lacunae. E: osteoclasts (arrows) excavating a resorption cavity (dashed line). F: osteoblasts (green arrow) refilling a remodeling cavity by producing osteoid (orange arrow). G: calcein-labeled mineralizing osteon; measurement of the distance between labels can be used to calculate the mineral apposition rate.
in various environments, including extreme environments, and “explaining the diversity of form and function of living organisms remains the ultimate goal of evolutionary biology, and the comparative method is one of its most powerful research tools” (94). Indeed, comparative approaches of animals adapted to particular niches have helped solve many biological and clinical problems. This comparative approach is known as the Krogh principle, after August Krogh who stated, “for such a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied” (66), where “problems” refers to physiological problems. When looking at specific traits and studying specific questions about the evolutionary physiology of bone, the comparative method can benefit by the inclusion of novel phylogenetically based analytical methods; however, these approaches have their own challenges and limitations (44).

**Ion Homeostasis Hypotheses for the Evolutionary Physiology of Bone**

The transition from sea to fresh water and the eventual transition to land most certainly were important adaptations that affected bone mineral content and physiology. Several hypotheses recognize that such large-scale environmental changes impacted overall physiology and affected the internal homeostasis of early vertebrates. Hypotheses outlining the potential adaptive processes of modern bone include the necessity to independently regulate physiological ionic homeostasis from the external environment (48, 49, 71, 102), store essential ions for continued physiological processes (50, 51, 90, 110), buffer the production of lactic acid with the evolution of more efficient and faster movements (96, 97), and provide an ionic waste system (11).

Fish have continuous access to calcium in water and regulate internal calcium levels primarily via the gills and the intestine (40), whereas many terrestrial vertebrates only occasionally ingest calcium. However, the plasma concentrations of total calcium in mammals (2.3–2.7 mM) and teleost fishes (2.5–3.0 mM) are similar and are maintained within a narrow range (116). The storage of minerals in the skeleton for use in ion homeostasis may have become more important in higher vertebrates (89). However, phosphorus concentration in salt and fresh water is low, and therefore fish rely on dietary phosphorus for normal skeletal mineralization (117). Furthermore, phosphorus deficiency can activate bone resorption in fish. Thus it is possible that bone first served as a phosphorus reservoir in fish. Bone resorption processes were already evolved as a tightly synchronized system before the transition to land, as evidenced in early heterostracan and osteostracan fishes (30, 51). Thus the physiological mechanism for storage and removal of phosphorus and calcium from the skeleton was already in place when animals took to land. The extracellular calcium-sensing receptor (CaSR) is found in many tissues (e.g., endocrine glands, intestine, kidney, and bone) of vertebrates and is implicated in playing critical roles in calcium storage and homeostasis. Evidence supports adaptive evolution of CaSR to accommodate changing needs in calcium storage and homeostasis as vertebrates moved from marine to fresh water to terrestrial environments, distinguishing terrestrial vertebrate CaSRs from aquatic vertebrate CaSRs (52).

**Mechanical Hypotheses for the Evolutionary Physiology of Bone**

Functional hypotheses of bone (i.e., those in which mechanical factors influence bone physiology) can be tested by comparing the structure of fossils and bones with those of extant animals. Some of the first evidence of phosphatic mineralization appeared as microscopic sclerites or dermal spines, which are homologous to early vertebrate tubercles (31, 86). Mechanical integrity of these spines may have provided a protective function against predators, as seen in some
Evolutionary Relationships and History of Bone Cells

The earliest evidence for mineralized tissues within the vertebrates is found in the feeding structures of the eel-like Conodonta (Table 1; FIGURE 4) (34, 36, 107), suggesting that the origins of hardened mineralized structures likely functioned in a feeding context rather than for skeletal support. Mineralized bone likely derived from acellular aspidin, which first appears in the Pteraspidomorphi, and specifically the Heterostraci (jawless fish with bony scales that formed dermal shields), as bony plates making up a dermal exoskeleton (Table 1; FIGURE 4). Galeaspids (extinct jawless fish with dermal bony head shield) exhibit the first evidence of a mineralized endoskeleton, but it is not until the Osteostraci (bony armored, jawless fish) that osteocyte-containing bone is observed in the fossil record (36). Heterostraci and Osteostraci both show evidence of bone resorption (30, 51), but the most conclusive evidence of secondary osteonal remodeling, as recognized histologically in extant species, is in Placodermi (extinct jawed fish with bony head armor) (36, 37, 58). In these fish, osteocyte lacunae are abundant, and secondary osteons are readily identifiable (FIGURE 5), similar to those histologically observed in living species (FIGURE 1) (37, 58). Furthermore, Placodermi are the first to develop all four endoskeletons (appendicular, axial, neurocranium, and splanchnocranium), similar to more derived vertebrates (35). Endochondral bone is thought to have appeared sometime between the Osteostraci and the Acanthodians (an extinct group of fish similar to sharks with both bony and cartilaginous features) (36) and is most representative in the Osteichthyes (bony fish) but lost in the cartilaginous Chondrichthyes (FIGURE 4) (35, 103). The requirement for bone growth, resorption, and repair could have been mediated by a shift from filter feeding to predatory feeding marked by significant adaptations appearing in the early Gnathostomata (jawed vertbrates) head (43). Surprisingly, although osteocytes are abundant in extant tetrapods and have important physiological roles in calcium (119) and mechanical homeostasis (61), there has been repeated losses of cellular bone and shifts to acellular bone (i.e., bone without osteocytes; although it does contain osteoclast and osteoblast cells) in several Teleost fish groups (FIGURE 4) (25). For example, the loss of osteocytes is widespread among most species making up the Neoteleosti (e.g., Acanthopterygii, Paracanthopterygii) and is also seen in a smaller number of species within the Clupeomorpha and Ostariophysi (FIGURE 4).
Although there appears to be few mechanical consequences, the transition from cellular to acellular bone does result in changes to the mineral content and the density of bone (25). The environmental conditions favoring such transitions remain obscure, but as the taxonomic relationships between these fish groups are better resolved and more data are generated on cellular vs. acellular bone, the

### Table 1. Summary of mineralized tissues in some aquatic vertebrate fossils

<table>
<thead>
<tr>
<th>Group</th>
<th>Period (Million Years Ago, Ma)</th>
<th>Habitat</th>
<th>Mineralized Elements</th>
<th>Tissue</th>
<th>Cellular Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conodonta (34) (eel-like chordate)</td>
<td>Upper Precambrian-Triassic (541-201)</td>
<td>Marine</td>
<td>Endoskeleton (?): mineralized feeding apparatus with odontodes; oro/pharyngeal denticles; splanchnocranium (?)</td>
<td>Phosphatic cones, enamel homolog (?); dentine (?); globular calcified cartilage</td>
<td>Acellular</td>
</tr>
<tr>
<td>Pteraspidomorphi (31, 86) (jawless armored fish)</td>
<td>Early to Middle Ordovician (485-488)</td>
<td>Marine</td>
<td>Dermoskeleton (fragmented): tubercles; mineralized scales; sclerites; dermal plates; bone attachment sites; globular calcified cartilage</td>
<td>Lamellar aspidin; isopedin; dentine (durodentine and orthodentine); enameloid</td>
<td>Acellular; spongy, tri-layered architecture with vascular canals; odontoblasts*; aspidinoblasts*</td>
</tr>
<tr>
<td>Heterostraci (50, 51) (jawless fish with bony scales that formed dermal shields)</td>
<td>Ordovician to Upper Devonian (485-350)</td>
<td>Marine, Estuary</td>
<td>Dermoskeleton: cephalothoracic plates; overlapping scales; tubercles; denticles</td>
<td>Aspidin; dentine; enameloid</td>
<td>Aspidones (osteons); aspidinocytes (?); aspidinoblasts*; aspidinoclasts*</td>
</tr>
<tr>
<td>Galeaspids (36, 114) (jawless fish with bony head shield)</td>
<td>Lower Silurian to Upper Devonian (442-372)</td>
<td>Marine</td>
<td>Endoskeleton: head shield and arculaia; neurocranium (juxtaposed to exoskeleton); globular calcified cartilage</td>
<td>Galeaspedin (similar to isopedin); bone that is of spherulitic and lamellar mineralization; globular calcified cartilage</td>
<td>Acellular</td>
</tr>
<tr>
<td>Osteostraci (30, 35) (bony armored, jawless fish)</td>
<td>Middle Silurian to Upper Devonian (433-372)</td>
<td>Marine</td>
<td>Endoskeleton: axial skeleton composed of head shield and pectoral girdle; neurocranium; paired fins; globular calcified cartilage</td>
<td>Bone; dentine; mesodentine; isopedin; calcified cartilage; perichondral bone; enameloid; endochondral bone (?)</td>
<td>Osteocytes; osteoblasts*; osteoclasts*</td>
</tr>
<tr>
<td>Placodermi (37) (early jawed fish with head armor)</td>
<td>Mid-Silurian to Devonian (428-354)</td>
<td>Marine, freshwater</td>
<td>Endoskeleton: appendicular and axial skeleton; neurocranium; splanchnocranium; jaws</td>
<td>Bone; perichondral bone; dentine; semidentine; orthodentine; enameloid; endochondral bone (?)</td>
<td>Osteocytes; osteoblasts*; osteoclasts*</td>
</tr>
<tr>
<td>Osteichthytes (35) (bony fish)</td>
<td>Early Devonian (420-present)</td>
<td>Marine, estuary, freshwater</td>
<td>Endoskeleton: heavily skeletonized appendicular and axial skeleton; neurocranium; splanchnocranium; jaws; dermatocranium</td>
<td>Endochondral bone; perichondral bone; bone; dentine</td>
<td>Osteocytes; osteoblasts; osteoclasts</td>
</tr>
</tbody>
</table>
Hormonal Influences on Bone Remodeling

Myriad circulating growth factors and hormones influence bone modeling and remodeling, and the evolutionary history of these molecules and their receptors may provide insight into the evolutionary physiology of bone. The role of only a few hormones will be discussed briefly to provide some examples. Estrogen is one of the most studied hormones in bone metabolism given its role in postmenopausal osteoporosis in humans. In humans, elevated circulating estrogen at puberty is associated with promoting calcium storage in the female skeleton in anticipation of high calcium demands during pregnancy and lactation (57). Decreased circulating levels of estrogen at menopause directly affect osteocytes, osteoblasts, and osteoclasts, leading to increased bone remodeling, with bone resorption outpacing bone formation and therefore leading to bone loss and increased fracture risk (64). It is important to note estrogen also has important effects on the male human skeleton (63). Estrogen receptors in extant vertebrates evolved from an ancient steroid receptor, which appeared before a protostome/deuterostome ancestor (38). Estrogen appeared later, before the appearance of bone, some
time before the cephalochordate/vertebrate ancestor. Therefore, estrogen binding to its receptor may have occurred by molecular exploitation by taking advantage of the ancient receptor’s promiscuous activity to create a new signaling pathway (38).

Parathyroid hormone (PTH), vitamin D, and calcitonin work in concert to maintain serum calcium and phosphate concentrations by influencing intestinal absorption of dietary calcium and phosphate, reabsorption of these ions in the kidneys, and calcium and phosphate exchange between bone and blood by bone remodeling processes. Phylogenetic analyses of PTH indicate that zebrafish have two PTH peptides that resulted from gene duplication following the evolutionary divergence between fishes and mammals (45). Interestingly, the zebrafish PTH peptides activate the human PTH receptor with high potency. It was suggested that fishes have a more complex PTH system than mammals and that PTH may have physiological roles in fishes beyond calcium regulation. A common ancestor of lobe-finned and ray-finned fishes are believed to have had three Runx proteins capable of interacting with the vitamin D receptor (70). After diverging from amphibians, Runx-2 and the vitamin D receptor became co-expressed to influence osteoblast differentiation in the amniote lineage. Additionally, the calcitonin receptor is highly conserved between fishes and mammals (77). Further investigation into the evolution of hormonal signaling in bone may increase our understanding of its role in modern bone physiology and our ability to treat metabolic bone diseases like postmenopausal osteoporosis.

**Calcium and Mechanical Challenges in Extant Vertebrates**

Understanding how living species deal with skeletal calcium and mechanical challenges provides insight into the evolutionary physiology of bone. Indeed, the dynamic environments that species inhabit and the diverse life histories of those species have resulted in various adaptations in bone physiology to help maintain calcium and mechanical homeostases. Regardless of the first physiological functions of bone, it is clear that bone physiological processes have adapted to serve mechanical and ionic functions in skeletal homeostasis for the diverse environments and lifestyles of living animals. However, without modern phylogenetic statistical analyses, we do not fully understand how these extant species came to have these unique bone physiologies, and thus one might consider them to be a collection of “Just So Stories.” Notwithstanding, these comparative stories are insightful in understanding how biological complexity evolved (94), and we hope they will inspire future phylogenetic and cladistics analyses to address specific questions in evolutionary bone physiology. Below are just a few examples of unique bone physiological processes. Many more have been described or are waiting to be described.

**Swimming vs. Gait**

Terrestrial animals have limited, periodic intake of calcium through diet, unlike the constant supply of calcium available to aquatic vertebrates. The first terrestrial animals were still dependent on aquatic environments for successful reproduction and offspring development, further suggesting that the evolution of the skeleton in regulating mineral homeostasis was likely a secondary trait to its mechanical functions (69). Lungfish are considered semiterrestrial animals because they can breathe air to survive periods of drought; however, these fish cannot mechanically walk. The different mechanical aspects of locomotion on land (e.g., gait) vs. in water (e.g., swimming) includes resisting gravity by supporting the weight of the body, providing protective structures to the soft-tissue organ compartments, and successfully navigating terrestrial obstacles. Locomotion on land is metabolically efficient despite the extra role of supporting the body (73). One of the first terrestrial vertebrates, Icthyostega (~363 Ma), used a sprawling stance during locomotion and required an aquatic environment for reproduction and development of its young (21). Early reptiles were the first animals to evolve the amniote egg, making them truly terrestrial despite their semi-aquatic lifestyles (e.g., crocodilian forms; Ref. 21). Thus the protective and
mechanical aspects of the skeleton afforded early terrestrial species the opportunity to expand into novel terrestrial niches.

**Targeted Bone Remodeling for the Repair of Mechanical Fatigue Damage**

Bone modeling and remodeling help maintain mechanical homeostasis by adjusting the size of bones to keep mechanical strains within a narrow range for the level of physical activity experienced by the animal (42, 98). If bone strain becomes too high, the risk of mechanical fatigue failure exponentially increases (22, 72). If bones are so large that mechanical strains are very low during routine physical activity, then the animal expends unnecessary metabolic energy (i.e., ATP for muscular contraction) to move the heavy skeleton during locomotion. A gracile bone reduces metabolic cost and can withstand high repetitive strains because bone remodeling can repair fatigue damage and limit damage accumulation (73). A decrease in normal mechanical loading on bones leads to unbalanced bone resorption and formation, which leads to increased intracortical porosity and decreased cortical thickness (99). This is likely due to the necessity for a light skeleton, which would promote agility and conserve metabolic energy (74). Indeed, bone strain rarely exceeds 3,000 microstrain in a wide range of animals (e.g., fish, turkey, horse; Ref. 98). Furthermore, bone remodeling maintains mechanical fatigue damage homeostasis (16, 112) through a process in which osteocytes detect fatigue microdamage and initiate the repair process by signaling to osteoclasts (61). This is done through osteocytic production of RANKL (62, 120, 121). If fatigue damage accumulation exceeds repair, stress fractures occur, as is common in runners and military cadets (74, 75). It has been suggested that fatigue damage repair by bone remodeling to prevent fracture is more important in the evolution of the skeleton than remodeling for mineral homeostasis (73, 74).

**Bone Remodeling in Acellular Fish Bone**

Like mammals, amphibians, and reptiles, the bones of basal teleost (e.g., carp and salmon) contain osteocytes, osteoblasts, mononucleated osteoclasts, and multinucleated osteoclasts. However, more advanced teleost (e.g., perches, cods, and their allies) have acellular bone (containing osteoblasts and osteoclasts, but not osteocytes; **FIGURE 4**), which contains primarily mononucleated osteoclasts and rarely, if ever, multinucleated osteoclasts. It was proposed that osteocytes may promote the formation of multinucleated osteoclasts and that the evolutionary disappearance of osteocytes led to advanced teleost bone containing predominantly mononucleated osteoclasts (116). It was also suggested that mononucleated osteoclasts are the more primitive bone resorbing cells, and their physiological role in humans and other mammals may be overlooked today (116).

Although osteocytes have a putative role in regulating calcium metabolism and bone adaptation to mechanical loading (29), acellular fish bone is capable of responding to calcium and mechanical challenges. For example, when calcium is depleted from ambient water, fish with acellular bone are able to suppress the activity of osteoblasts and mobilize calcium from the endoskeleton using mononucleated and multinucleated resorbing cells (106). There are also some provocative observations suggesting acellular teleost bone exhibits bone modeling responses due to altered mechanical loading, despite the lack of osteocytes (100). Mechanical loading increased mineral apposition rate similarly in fish with cellular and acellular bone. Furthermore, there is histological evidence of bone remodeling in the acellular bone of the bill of blue marlin (**FIGURE 6**) (100). Since this region of the bill is subject to high cyclic mechanical loading, bone remodeling may serve to repair fatigue damage in acellular bone as it does in the cellular bone of terrestrial vertebrates. However, terrestrial vertebrates appear to use fatigue damage-induced apoptotic osteocytes as beacons for osteoclasts to initiate targeted remodeling (112). How could teleost with acellular bone regulate modeling and remodeling when tetrapods seem dependent on osteocytic regulation of modeling and remodeling? Possibly by using osteoblasts instead of osteocytes as the mechanical sensors and regulators of modeling and remodeling (100, 116). Furthermore, why did different teleosts evolve to lose the osteocyte? Possibly because depletion of dietary and ambient calcium is an unlikely circumstance for advanced teleost, eliminating the need for osteocytic osteolysis for calcium homeostasis and because acellular bone can still model and remodel to maintain mechanical homeostasis (100). This would be advantageous from a metabolic energy perspective as the need for osteocytic osteolysis for calcium homeostasis and because acellular bone can still model and remodel to maintain mechanical homeostasis (100).
results in the production of lactic acid. The ability for fast movement likely first arose with the evolution of early vertebrates and may actually have been a selecting force in their less-soluble, calcium-phosphate-composed hard tissues (97). Not only were these hard tissues contributing mechanically as early attachment sites and levers for the muscular system, but they also may have provided mechanisms for buffering lactic acid production. In addition to anaerobic muscle activity, lactic acid is also produced in conditions of low oxygen availability, such as in diving freshwater turtles (*Chrysemys picta bellii*) (55). Turtles have specialized dermal bone plates covered by keratinous scutes collectively known as the turtle's shell or carapace. This structure, not including the rest of the skeleton, contains over 95% of the body's calcium and phosphate (54). Surviving prolonged anoxic submersences and subsequent build-up of lactic acid in turtles is attributed in part to the buffering and storage of lactic acid by the shell and skeleton (54).

**Antlers**

The growth of antlers, dermal appendages of the skull in members of the deer family (*Cervidae*), may require calcium to be mobilized from the skeleton. These fast-growing bony structures (averaging 2.15 μm/day in red deer, *Cervus elaphus*; Ref. 47) are important for mate selection (4, 92). The rapid mineral deposition and growth of antlers suggests that large sources of calcium and phosphate (either dietary or skeletal) are required for their development. Surprisingly, although other dietary minerals fluctuate in antlers, the calcium composition of antlers does not change significantly with diet (19, 39). Rather, antler growth results in mobilization of skeletal calcium stores (3, 105), and bone resorption occurs in the long bones, ribs, and other skeletal elements (2, 83). White-tailed deer (*Odocoileus virginianus*) mobilize calcium from the skeleton by increasing parathyroid hormone (PTH) levels during early antler growth in the spring and the final stages of antler mineralization (24). Calcitonin appears to help protect the skeleton from excessive bone resorption.

Antlers may also contribute to calcium homeostasis. Osteocytic osteolysis has been observed in antlers (6, 7) and occurs during the period of fastest antler growth and remodeling in reindeer (*Rangifer tarandus*) of both sexes (7). Osteocytic osteolysis continues in the antlers during winter, albeit much reduced, when food is limited. This potential calcium store may be particularly important in pregnant reindeer up to the time of parturition when the antlers are shed. Thus, although diet does not affect calcium composition of the antlers themselves, antlers may contribute minerals during seasonal reproductive and dietary challenges in reindeer (7). Although physiologically interesting, the contributions of antlers to the mineral homeostasis of the cervid skeleton is likely a secondary trait to the primary role of antlers in mate selection and reproduction. Antler is less mineralized than long bones of the endoskeleton, which makes antler less stiff, but capable of absorbing large amounts of energy before failure (i.e., antler is tougher than endoskeletal bone) (28). This is advantageous for the mechanical function of antler: fighting during the rut.

**Mammalian Pregnancy and Lactation**

Pregnancy and lactation indeed pose prominent challenges for mammals in regulating internal calcium balance and skeletal quality. Calcium is essential for a healthy neonate, and it is obtained through increased maternal intestinal absorption during early pregnancy and through mobilization of calcium from the skeleton during late pregnancy (27). Furthermore, the fetus maintains a high serum calcium concentration compared with that of the mother throughout pregnancy, the significance of which remains unknown (65).

Mammary glands and the ability to lactate are derived adaptations of mammals (~200 Ma),...
which are tightly linked to the skeleton (108). The osteoblast-derived protein RANKL signals to osteoclast progenitors to differentiate and increase bone resorption, thereby releasing calcium into the extracellular solution. Mammary epithelial cells also express RANKL during pregnancy, and RANKL contributes to the formation of the lactating mammary gland (108). Additionally, casein is a specialized secretory calcium-binding phosphoprotein (SCPP) found in milk. This protein functions to supersaturate milk with calcium-phosphate, contributing to infant skeleton and tooth development (60). Furthermore, lactating mice have been shown to liberate calcium from the skeleton through osteoclastic bone resorption processes (93). Increased bone resorption during lactation contributes to decreased bone strength in rats, which could be restored postlactation (111). In humans, prolonged breastfeeding increases risk of vertebral fracture (14).

Avian Medullary Bone

Evidence suggests that mammary glands evolved from specialized apocrine-like glands associated with hair follicles of parchment-shelled, egg-laying synapsids (87, 88). This adaptation not only ensured the hydration of the egg (87) but may also have allowed the evolution of decreased egg size and eventual altricial hatching of offspring with increasingly nutrient-rich mammary excretions (i.e., milk; Ref. 88). The production of calcium-carbonate shells of avian eggs (primarily studied in Gallus gallus; Ref. 53) puts a toll on mineral homeostasis and the skeleton. The marrow, or medullary, cavity of adult birds changes drastically with their reproductive cycle. During the preovulatory period, bone is rapidly laid down by osteoblasts in the medullary cavity (12, 13). Postovulation, dramatic resorption of this medullary bone commences, and the serum concentrations of calcium are elevated to hypercalcemic levels (12, 13). Resorption of the medullary bone is carried out by osteoclasts, which release calcium from the skeleton to contribute to the formation of the shell of the egg (84). It is estimated that 25–40% of the calcium required for the shell is obtained from this medullary bone (26).

Hibernation

Hibernating animals (e.g., bears, woodchucks, and ground squirrels) have evolved numerous unique physiological traits that allow them to survive extreme environmental conditions. These traits include maintenance of the skeleton and preservation of calcium homeostasis despite decreased mechanical loading on bones during physical inactivity, anorexia, and in some cases anuria, for up to 9 mo each year. During hibernation, hibernators decrease metabolic rate and body temperature to conserve energy (20, 109). Bone remodeling is reduced to ~25% of summer levels in hibernating bears (82), similar to overall reductions in metabolism (109). Unlike in humans and other animals, bone formation and resorption remain balanced in bears and large rodent hibernators during physical inactivity (32, 81, 118). Balanced bone remodeling maintains the concentration of plasma calcium at normal levels in hibernating bears, despite the fact they are anuric (41). Furthermore, bear cubs born during hibernation are small (~0.5–1 lb.), which is ~1/30th the ratio of birth weight to maternal weight of other eutherian neonates (67). Low birth weight in bears may help to protect the maternal skeleton by reducing the need for calcium to be liberated from bone during this period of fasting. Indeed, the mechanical properties of bear bones are not different before and after hibernation despite nearly 6 mo of physical inactivity, pregnancy, and lactation (80).

Some small hibernators, such as ground squirrels (79) and hamsters (104), show evidence of osteocytic osteolysis during hibernation, whereas some larger hibernators like bears (78) and marmots (118) show that osteocyte lacunae become smaller during hibernation, suggesting the role of osteocytic osteolysis in calcium homeostasis in hibernators may be a function of animal size (32). Larger hibernators may have sufficient trabecular bone surface to maintain calcium homeostasis via bone remodeling, albeit reduced during hibernation, whereas smaller hibernators may need to utilize the additional surface area found in osteocyte lacunae.

Conclusions

Calcium, a critical ion in all cellular life, has helped provide the means for exploitation of new habitats by contributing to diverse skeletal structural adaptations, which promote calcium and mechanical homeostases in extant vertebrates. This is accomplished via osteocytes and bone remodeling in terrestrial vertebrates. Basal teleosts also use osteocytes and bone remodeling for bone mechanical homeostasis, and maybe for calcium
homeostasis, but they have other sources (i.e., water and exoskeleton) of calcium available that are likely the primary sources. There has been much focus on bone physiology for calcium homeostasis. However, given calcium, but not phosphorous, is abundant in sea water, where bone first appeared, it is possible that an early role for bone and bone physiological processes was to serve as a reservoir for phosphorus. Advanced teleosts have some ability for skeletal phenotypic plasticity in response to altered mechanical environments despite lacking osteocytes. Studies in fish indicate bones of individuals can adapt to the mechanical environment without the aid of osteocytes and that mononucleated osteoclasts play important roles in bone resorption. Studies on osteocyte-independent mechanisms of mechanical adaptions in bone and the role of mononucleated osteoclasts in terrestrial vertebrates may be warranted. The evolutionary physiology of bone informs our understanding of the skeletal diversity in extant vertebrates and may provide clues for possible treatments for skeletal diseases, such as osteoporosis.

No conflicts of interest, financial or otherwise, are declared by the author(s).


References


