The Aging of Wolff's "Law": Ontogeny and Responses to Mechanical Loading in Cortical Bone

Osbjorn M. Pearson[1]* and Daniel E. Lieberman[2]

[1]*Department of Anthropology, University of New Mexico, Albuquerque, New Mexico 87198-1086*
[2]*Peabody Museum, Harvard University, Cambridge, Massachusetts 02138*

KEY WORDS ontogeny; senesence; Haversian remodeling; modeling

ABSTRACT The premise that bones grow and remodel throughout life to adapt to their mechanical enironment is often called Wolff's law. Wolff's law, however, is not always true, and in fact comprises a variety of different processes that are best considered separately. Here we review the molecular and physiological mechanisms by which bone senses, transduces, and responds to mechanical loads, and the effects of aging processes on the relationship (if any) between cortical bone form and mechanical function. Experimental and comparative evidence suggests that cortical bone is primarily responsive to strain prior to sexual maturity, both in terms of the rate of new bone growth (modeling) as well as rates of turnover (Haversian remodeling). Rates of modeling and Haversian remodeling, however, vary greatly at different skeletal sites. In addition, there is no simple relationship between the orientation of loads in long bone diaphyses and their cross-sectional geometry. In combination, these data caution against assuming without testing adaptationist views about form-function relationships in order to infer adult activity patterns from skeletal features such as cross-sectional geometry, cortical bones density, and musculoskeletal stress markers. Efforts to infer function from shape in the human skeleton should be based on biomechanical and developmental models that are experimentally tested and validated. Yrbk Phys Anthropol 47:63–99, 2004.

Paleontologists, archaeologists, and anthropologists routinely rely on skeletal material to make inferences about a wide range of subjects including taxonomy, evolutionary relationships, life history, behavior, and so on. To make such inferences reliably (or, even better, to test them), it is necessary to understand many details of the complex array of genotypic and environmental influences on the development of skeletal phenotypes. To what extent are the variations in bony shape that we observe the result of genetic vs. environmental influences? And which ones? As frequently noted, variations that have a strong genetic basis are most useful for testing systematic hypotheses, whereas variations that are substantially affected by interactions between their mechanical environment and their genotype (epigenetic interactions) are more useful for inferring habitual behaviors (e.g., Lieberman, 1999; Lovejoy et al., 1999).

Despite its importance, the task of determining to what extent variations in the skeleton derive from particular genetic or epigenetic influences is not simple. The bad news is that the developmental bases of skeletal variations are too often obscure because the processes that generate and alter bones are themselves complex. The good news, however, is that this complexity is increasingly appreciated and tractable. Our approach to the skeleton has become less naive (but more arduous) in many respects, thanks to several theoretical advances along with an explosion of new data on bone developmental genetics and biomechanics. Three trends deserve special mention. The first is the decline of the "adaptationist paradigm," in which form is assumed a priori to be related to function (the so-called "argument from design"). Since the influential "spandrels" paper of Gould and Lewontin (1979), it has become much less common to assume that a given aspect of morphology represents an adaptation, defined as a heritable variation that was selected for because it improved an organism's survival or reproductive success. Instead, morphologists now routinely evaluate arguments from design in terms of performance criteria with respect to a biomechanical model (Lauder,

Grant sponsor: NSF; Grant number: IBN 96-03833; Grant sponsor: American Federation for Aging Research; Grant sponsor: George Washington University; Grant sponsor: Harvard University; Grant sponsor: University of New Mexico.

*Correspondence to: Osbjorn M. Pearson, Department of Anthropology, University of New Mexico, MSC01 1040, Albuquerque, NM 87198-1086. E-mail: ompear@unm.edu

DOI 10.1002/ajpa.20155
Published online in Wiley InterScience (www.interscience.wiley.com).

1996) or in terms of alternative developmental pathways. As a result, many features have lost their status as "adaptations." For example, in spite of recent arguments that cranial vault thickness in *Homo* is an adaptation to resist blows to the head (Boaz and Ciochon, 2004), vault thickness is not only highly variable and epigenetic (Lieberman, 1996), but also may have little effect on the performance of the cranium in response to loading, given its domed shape that efficiently distributes stresses over large areas (Demes, 1985; Skerry, 2001). Similarly counfounding processes also affect interpretations of postcranial skeletal morphology. For example, Pearson (2000) found that ratios of articular width relative to bone length were closely correlated among long bones throughout the skeleton (with the exception of the clavicle) and that these ratios were also correlated, albeit less closely, to conventional indices of diaphyseal robusticity. Much of the strength of these correlations appears to stem from a single variable, body mass, which affects the mechanical environment of all of the limb bones during development (van der Meulen et al., 1993).

The second trend is the recent explosion of knowledge about skeletal genetics and biomechanics. The last decade has witnessed fundamental advances in our understanding of the genes that regulate skeletogenic cells such as osteoblasts, osteoclasts, and chondrocytes (Karsenty, 1999, 2003; Olsen et al., 2000); on how bones sense and tranduce strain (Duncan and Turner, 1995; Ehrlich and Lanyon, 2002); and on the mechanical properties of bones and their effects on bone growth and function (Martin et al., 1998; Carter and Beaupré, 2001; Currey, 2002). As a biological material, bone is becoming much less of a black box than it used to be.

The third trend to consider is the growing importance of evolutionary developmental biology ("evo-devo") in biological anthropology (e.g., Lovejoy et al., 1999, 2003; Chiu and Hamrick, 2002; Hlusko et al, 2004; Lieberman et al., 2004b). As a field, evo-devo explicitly examines evolutionary transformations of the processes by which the genotype generates phenotype (for reviews, see Hall, 1999; Carroll et al., 2001; Wilkins, 2002). Evo-devo approaches not only provide key insights into developmental processes of interest (e.g., patterning of the skeleton), but also highlight the degree to which features in the skeleton are intrinsically integrated at different hierarchical levels of development. Advances in this field have rendered untenable the assumption that one can atomize the skeleton into discrete, supposedly independent traits, each with its own adaptive story (West-Eberhard, 2003). Instead, we need to consider how shared developmental pathways may lead to covariation and correlation throughout the phenotype, leading us to reexamine how we interpret many skeletal variations. For example, one can identify many changes in craniofacial form between modern *Homo sapiens* and archaic *Homo* that have been assumed explicitly or implicitly to indicate independent selection on adaptations for cognition, language, locomotion, or diet (reviewed in Wolpoff, 1999). However, it seems increasingly likely that many derived modern human features are an integrated package whose appearance may result from a small number of developmental shifts, possibly in brain shape, that occurred early in cranial ontogeny (D.E. Lieberman et al., 2002, 2004b; Ponce de Leon and Zollikofer, 2001; Krovitz, 2003). If so, then many, if not most derived features of the modern human craniofacial skeleton (e.g., retracted faces, domed frontal bones) may be by-products ("spandrels") of more fundamental shifts rather than adaptations that have been selected in their own right.

Because many biological anthropologists focus primarily on bones or fossils, the above-described advances in bone biology, along with a renewed interest in bone development and function, have led to some vastly different opinions about how to interpret the functional bases of many skeletal variations. Some anthropologists have become increasingly focused on the role of patterning genes in causing phenotypic variations in the skeleton, sometimes downplaying the role of mechanical loading on bone shape and structure (e.g., Lovejoy et al., 2003). For example, Lovejoy et al. (1999) hypothesized that many differences in pelvic shape between chimpanzees and australopithecines were caused entirely by changes in a hypothetical set of pattern-generating genes that would alter the superior-inferior growth of the chimpanzee pelvis. Other biological anthropologists have focused more explicitly on the epigenetic role of the skeleton in responding to mechanical loading, either testing or assuming a relationship between skeletal form and its biomechanical function. These studies fall into four general categories: 1) cross-sectional geometry (e.g., Ruff, 2000b, 2002; Polk et al., 2000; Lieberman et al., 2004a); 2) joint form and pathology (e.g., Hamrick, 1999; Jurmain, 1999; Lieberman et al., 2001); 3) trabecular architecture (e.g., Macchiarelli et al., 1999; Fajardo and Müller, 2001; Ryan and Ketcham, 2002; Richmond et al., 2004); and 4) histology (e.g., Schaffler and Burr, 1984; Burr et al., 1990; Lieberman et al., 2003).

A complete review of genetic vs. environmental influences on bone morphology is beyond the scope of this paper. Instead, we review the particularly important problem of the extent to which bones adapt to their mechanical environment over time (Roux, 1881), potentially leading to a predictable relationship between structure and function. This proposition, often described as "Wolff's law" today (see below), actually incorporates three concepts inherited from 19th century anatomists: bone is deposited and resorbed to achieve an optimum balance between strength and weight, trabeculae in cancellous bone tend to line up with the directions of principal stresses that they experience, and both phenomena occur through self-regulating mechanisms that respond to mechanical forces acting upon bone tissues

(Martin et al., 1998). Wolff's contribution to the "law" consisted of the proposal that trabecular bone tended to be formed during growth and development in orientations that corresponded to principal mechanical stresses that acted on the bone, and that hypothetical mathematical laws could explain this process (Wolff, 1986; Martin et al., 1998).

Within recent decades, Wolff's "law" has been frequently invoked in spite of being widely critiqued on a number of grounds (e.g., Bertram and Swartz, 1991; Cowin, 2001; Currey, 2002; Lovejoy et al., 2003). Given the predominance of cortical bone in the fossil record, we focus on several key topics relevant to interpreting variations in cortical bone, particularly in diaphyses. Epiphyses and articular surfaces also undoubtedly respond during growth to mechanical forces (Frost, 1979, 1994, 1999; Hamrick, 1999; Plochocki and Organ, 2003; Plochocki et al., 2004), but the mechanisms for these responses in chondroblasts and chodrocytes involve different mechanical stimuli and molecular pathways (Kronenberg, 2003; Harada and Rodan, 2003; Boyle et al., 2003); the interactions between age and loading in cartilage would merit a review in its own right. Mechanisms responsible for modeling and remodeling trabecular bone are also quite complex (although they involve the same physiological processes by which bone cells resorb or deposit new cortical bone) and merit separate, detailed treatment beyond the scope of this article.

We limit this review to mechanisms that act upon cortical bone. In particular, we first review the mechanisms by which cortical bone senses and transduces information about its mechanical environment to skeletogenic cells. We then review evidence for variable responses of bone to loading from the cellular to macroscopic levels, and the interaction between these processes and other factors that influence bone growth (e.g., hormones, genes, and mechanical stimuli) at different stages of ontogeny. Many of these issues were reviewed previously by Forwood and Burr (1993), but numerous subsequent studies provided much new information about the molecular control of bone apposition and resorption as well as changes during growth and development of the responsiveness of bone to its mechanical environment. Next, we critically assess the relationship between mechanical loading (principally various kinds and intensities of exercise in humans) on long bone cross-sections and bone mineral density. We conclude with some potential directions for future research.

BONE CELL RESPONSES TO MECHANICAL LOADS

The idea that a bone's shape is formed by interactions with its mechanical environment is often described as "Wolff's law." Although Wolff's law is frequently invoked, it is neither a law, nor completely true. Wolff (1892) originally formulated his "law" to explain how the the orientations of trabeculae in the proximal femur and elsewhere became established during ontogeny (Martin et al., 1998), but the concept has grown into a more general, organizing principle that seeks to provide a means for understanding the gross shapes and adaptations of bones. It is now common to use Wolff's "law" as a catch-all concept to denote the adaptation of bone to mechanical stimuli. In this more generalized role, the "law" has evolved into a black-box axiom of functional morphology to relate skeletal form and function (Fig. 1). However, the many flaws and exceptions to Wolff's "law" in both its particular and more general forms have rendered the term somewhat useless (for critiques, see Bertram and Swartz, 1991; Cowin, 2001). What use is a black box that is not even completely true? Despite a recent proliferation of theories on what factors modulate the responses of bones, it is probably best not even to use the term "Wolff's law." Instead, it is more useful to consider separately four related questions that together comprise the general phenomenon of bone responses to mechanical loading. 1) What are the mechanical problems that confront bones? 2) How do bones perceive forces? 3) How do applied loads induce osteogenic response by various cell types in different regions? 4) What factors modulate these responses to generate variations in relationships between structure and function?

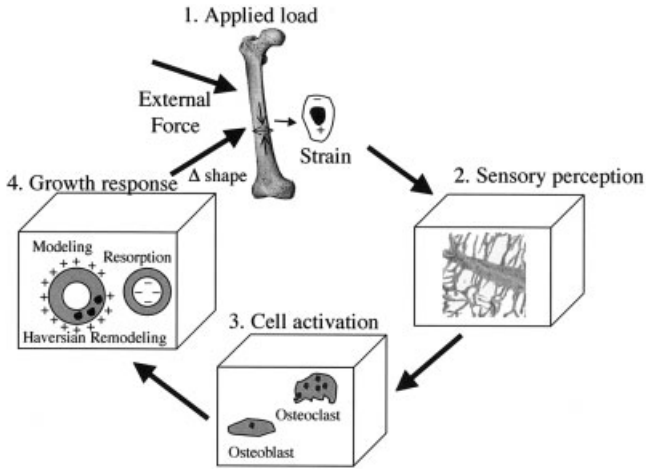

Fig. 1. Responses of bone to loading.

What are the mechanical problems that confront bones?

Bone is a complex tissue with multiple functions. The primary function of bone is to be stiff, i.e., to resist deformation in response to both internal (primarily muscular) and external forces (Currey, 2002). In addition, bone must also be sufficiently strong (resist breakage) in order to remain stiff. Bone strength can be increased in a number of ways, e.g., by adding bone mass, by changing bone geometry to redistribute the forces (stress) that it must resist, or by alterations of its microstructure via processes such as Haversian remodeling (Currey,

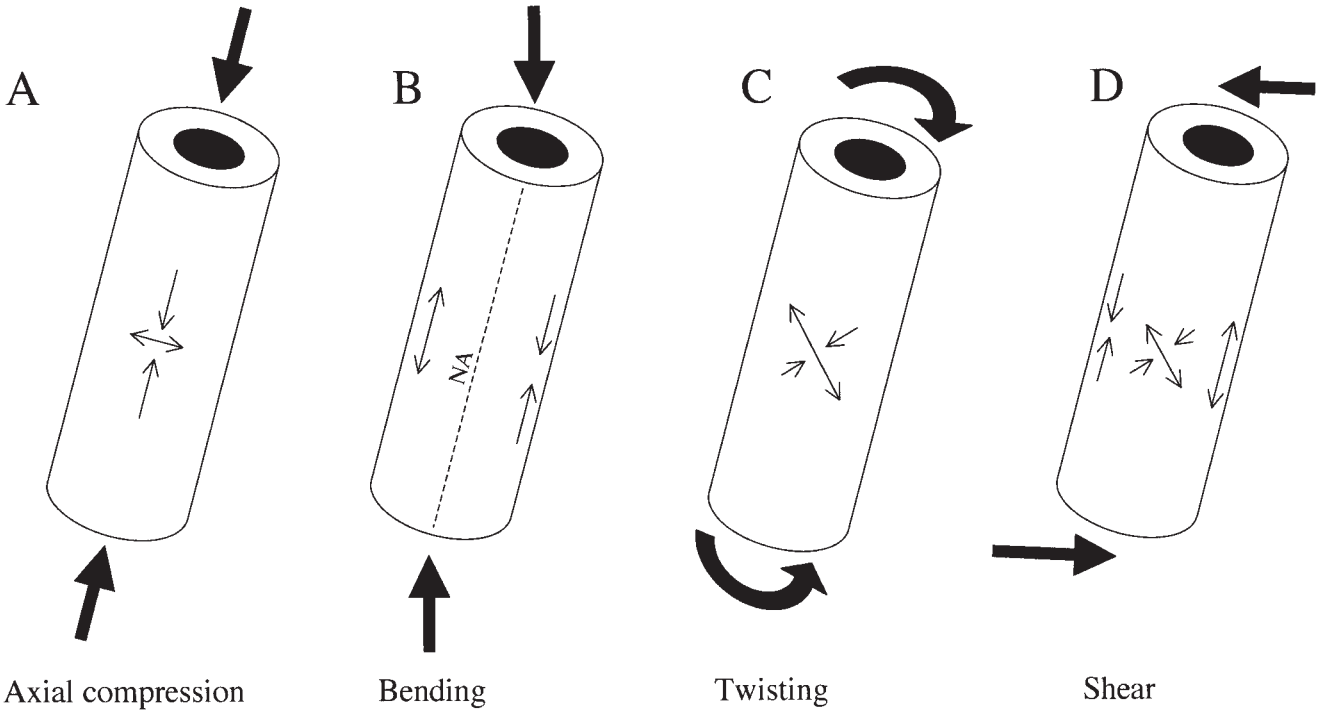

Fig. 2. Beam and cross-section views of applied forces and strain distributions in compression (**A**), bending (**B**), twisting (**C**), and shearing (**D**). Thick arrows indicate applied loads; thin arrows indicate resulting strains.

2002; Martin and Burr, 1989). Bones additionally serve as attachment sites for muscles, ligaments, and tendons, house various organs and spaces (chiefly marrow in the postcranium, and the nasopharynx, brain, and organs of sense in the skull), and act as a dynamic calcium reservoir. Furthermore, bones have to perform all these tasks during growth, permitting dramatic changes of size and shape in both fetal and postnatal life without any compromise in function. Because of these varied, complex, and changing roles, it is unsurprising that bone has evolved to be a highly integrated and dynamic tissue that is influenced by a wide range of genetic and nongenetic stimuli.

Given its primary function, any review of how and to what extent bones change in response to their mechanical environment needs to begin with a discussion of how bones remain stiff in response to applied loads. Loading derives from forces applied to a bone either from a muscle pulling on an origin or insertion region, or from external forces acting on a bone across a joint or from the outside world (e.g., the ground). From the bone's perspective, the two most important parameters are *stress* (σ, defined as force, F, per unit area, A) and *strain* (ϵ, defined as change in length, ΔL, per unit length, L). Simply put, force generates stresses of varying intensity, which produce strains of varying magnitude and mode. At a basic level, strain can be either tensile (positive elongation) or compressive (negative elongation), but in reality most bones experience some combination of the two, although a few bones such as the cannon bone of a horse can experience a loading regime that approaches pure compression (Gross et al., 1992). An applied stress generates orthogonal axes of compression and tension, whose ratio depends on the material properties of the tissue. Typically, bones are loaded in four possible ways, depicted in Figure 2: they can be compressed axially, bent, sheared, and twisted (Carter and Beaupré, 2001). Often these loads occur in combination (most long bones are mostly bent, but also compressed and twisted to varying extents).

Bone varies mechanically quite substantially in its resistance to applied forces in ways that derive from key aspects of the tissue's structure and composition (Currey, 2002; Carter and Beaupré, 2001; Martin et al., 1998). Bone is essentially a two-phase substance (like fiberglass or reinforced concrete): about 35% of a bone's weight is organic, comprised mostly of collagen fibers, along with a small percentage (<10%) of noncollagenous proteins; the other 65% of bone is largely a calcium-phosphate mineral known as hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, that contains traces of various impurities such as citrate, fluoride, and magnesium. Osteoblasts synthesize bone in a two-step process. First, they secrete an initial collagen matrix (osteoid), typically in an organized lattice (see below) that forms the basic histological framework of the tissue. Osteoblasts then mineralize the collagen by precipitating crystallites in the form of needles, rods, and plates within and between the collagen fibers. Collagen thus serves to organize bone tissue, influencing most aspects of its

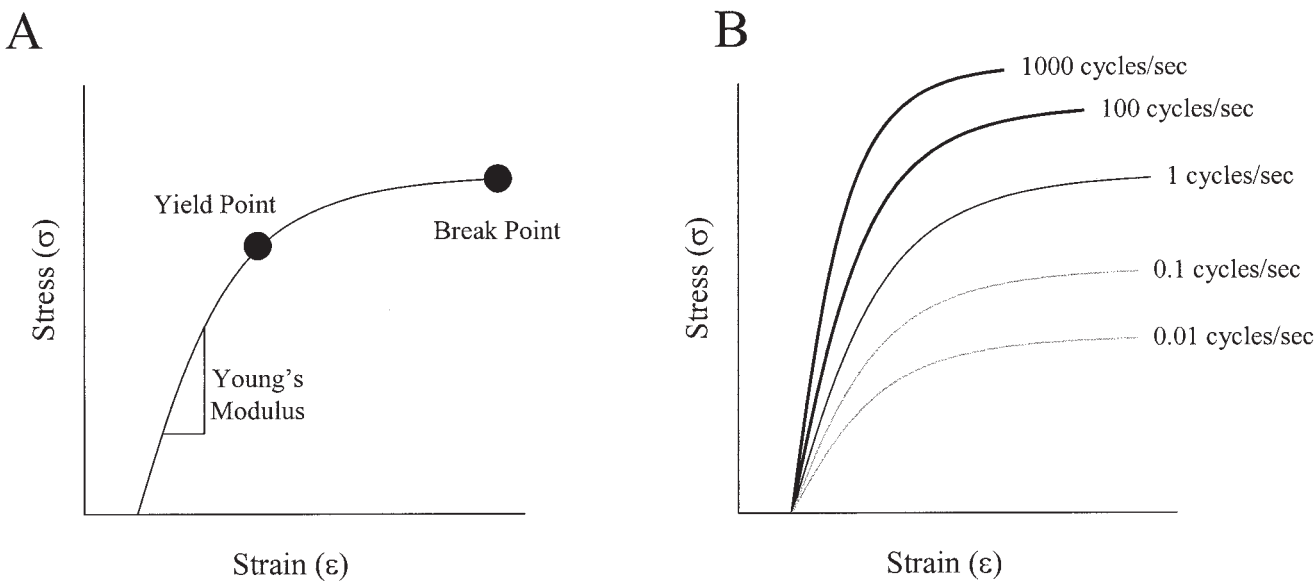

Fig. 3. **A:** Stress-strain curve for simple load. **B:** Stress strain curves at different numbers of loading cycles per second (Hz).

histology. The role of collagen in organizing the mineral component of bone, however, is quite complex, as demonstrated by Takano et al. (1996), who showed that bone crystals laid down in matrix along randomly oriented collagen fibers in newly formed woven bone initially lacked any difference in stiffness (anisotropy), but then gradually became anisotropic and more consolidated and mineralized after 16 weeks. This change appeared to have arisen due to a greater regularity of orientation in the hydrodyapatite crystals after 16 weeks, a change that had occurred without any accompanying change in collagen orientation. Collagen also provides bone with much of its elasticity and much of its ability to resist tension; cross-links between collagen molecules, especially in biochemically immature cross-links, also provide an important part of bone's toughness (Banse et al., 2002). Biochemically "mature" cross-links have an altered chemical structure (and mechanical properties) reative to newly formed cross-links (Banse et al., 2002). In contrast to collagen, bone mineral gives bones their stiffness and resistance to compression. Both bone mineral crystals and collagen undergo biochemical changes with age that diminish their capacity to provide the strength and toughness that bones need (Zioupos, 2001; Bailey et al., 1999; Bailey and Knott, 1999; Akkus et al., 2004).

In combination, collagen and mineral create a substance that has a high degree of both stiffness and strength in response to applied forces. Figure 3A shows a typical load-deformation curve for bone that illustrates some key mechanical properties. Note that at low stresses and strains, these two parameters have a linear slope, known as Young's modulus of elasticity (expressed in Pascals, *Pa*, defined as N/m^2). Within this range of loads, bone tissue properties are technically elastic because the bone returns to its original size and shape after loading has ceased. However, above a certain stress magnitude, known as the yield point, the bone responds plastically to applied stresses, permanently deforming up to a point at which it fractures. Note that bone loses most of its stiffness when plastically deformed; not surprisingly, during normal ontogeny, most bones grow large and thick enough to stay within the elastic region of the deformation curve (for further details, see Currey, 2002). Many intrinsic and extrinsic factors affect a bone's mechanical properties in response to loading. Intrinsic factors include the degree of mineralization (higher mineral density increases Young's modulus) and the organization of the tissue (e.g., porosity, orientation of collagen fibers, density, and histological structure; reviewed in Martin et al., 1998). Extrinsic factors include the mode of strain (bone is stronger in compression than tension), the duration of strain events, and the rate of strain. The latter factors are particularly interesting, because bone (like most materials) is partially viscoelastic. As strain rates increase, Young's modulus increases, often dramatically (as shown in Fig. 3B).

Young's modulus is a critical parameter that influences bone function because of the deleterious effects of strain on bone structure via creep and fatigue, both of which contribute to fracture damage. Cyclic stresses produce fatigue and promote creep, but creep can also happen in response to continuously applied loads, and tensile and compressive loads produce somewhat different effects (Carter and Caler, 1983, 1985; Caler and Carter, 1989). The mathematics of fracture damage are complex, but primarily derive from the concentrated intensity of an applied stress relative to the fracture toughness of the material (for a thorough review, see Martin et al., 1998). Typically, bones loaded within their elas-

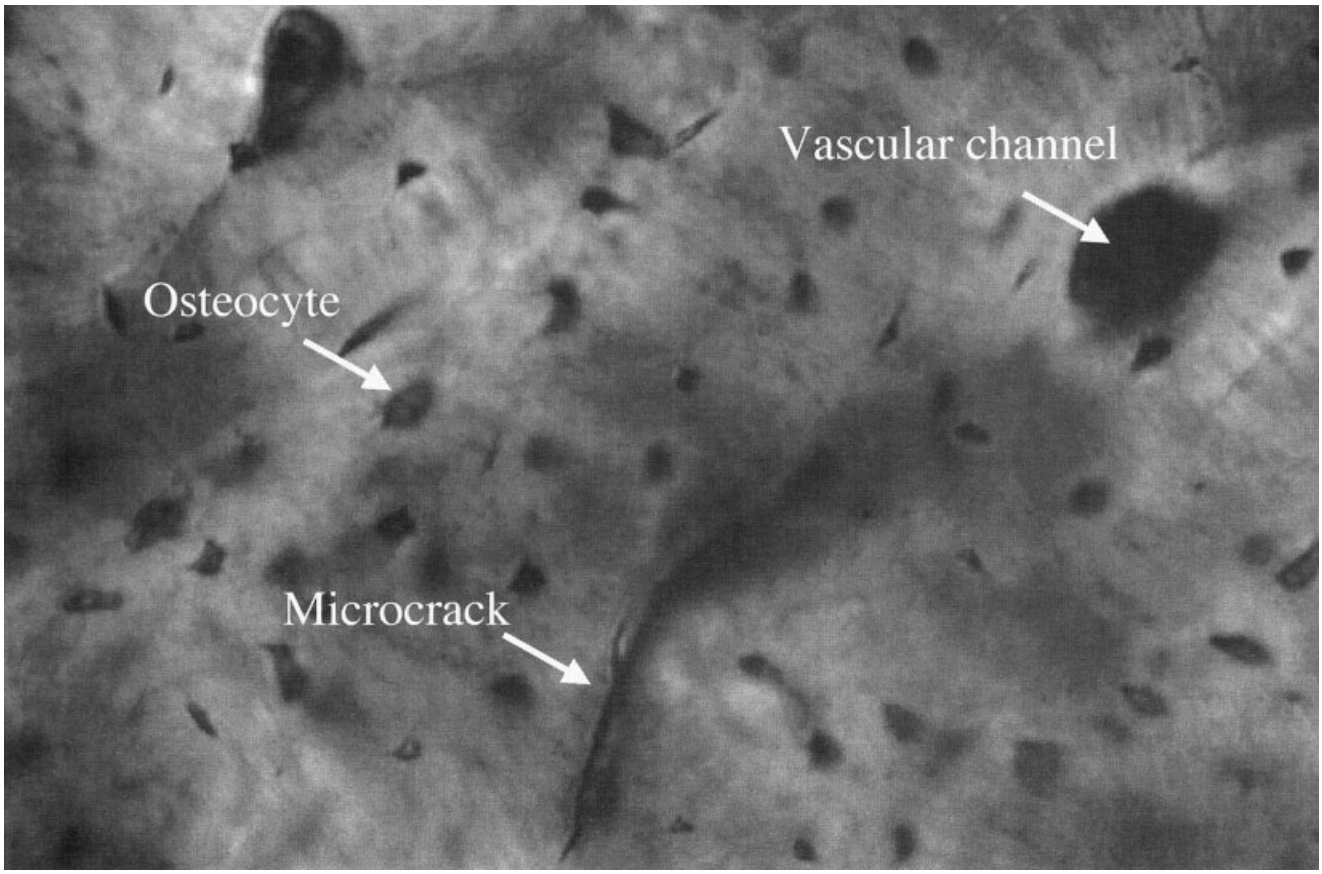

Fig. 4. Microcrack in sheep cortical bone, with crack, an osteocyte in its lacuna, and a vascular channel.

tic range at sufficient intensities become damaged through two independent processes. First, strains initiate microcracks (Fig. 4), whose size and rate of accumulation are primarily determined by the tissue's structure as well as by variations in applied stress magnitudes, strain rate, and number of cycles. Second, these cracks grow and propagate largely as a result of the structural characteristics of the tissue. The greatest potential danger of microdamage accumulation is that cracks can begin to coalesce at some threshold, rapidly weakening the tissue and leading to mechanical failure. Fortunately, this type of failure does not happen very often, particularly in compact bone. Although compact bone develops microcracks quite readily within normal ranges of loading, its complex lamellar organization is particularly good at limiting microcrack propagation by creating boundaries (primarily cement lines between lamellae) across which cracks cannot easily migrate (Currey, 2002). In addition, microcrack damage decreases bone stiffness, paradoxically making the bone less susceptible to further fracturing (Currey, 2002). This process is highly adaptive for bone because it increases toughness (the total amount of work needed to break a substance) and extends fatigue life (Vashishth, 2004; Vashishth et al., 1997).

How do bones sense loading?

The second stage in bone response to mechanical loading is mechanotransduction, the process by which cells sense mechanical stimuli in their external environment and then translate the information into a signal that can potentially elicit some response either within the cell or in another cell. Of all the black boxes within Wolff's "law," this stage is the least understood, and probably occurs through several mechanisms. The hypothesis that has received the most attention and support is that specialized osteoblast cells called osteocytes act as strain receptors and transducers (Marotti, 1996). Osteocytes (Fig. 4) are osteoblasts that become entombed throughout bone matrix during growth (their density may be partly a function of the tissue's growth rate). Osteocytes have as many as 80 long processes, termed canaliculi, that radiate out about 15 μm from the cell body in every direction. These processes communicate bidirectionally with those of other osteocytes via transmitter proteins (connexins) at specialized gap junctions (Cowin et al., 1991). Together, osteocytes throughout a bone form a fantastically complex connected cellular network (CCN) that includes cells along the periosteal and endosteal membranes which surround the external

and internal surfaces of the bone, respectively (Marotti, 1996; Cowin and Moss, 2001). In turn, these superficial osteocytes are connected to osteoblasts that line the bone surface in the periosteum, which in turn connect to preosteoblasts within the membrane (Jones and Bingman, 1991; Civitelli, 1995; Marotti, 1996). In effect, the CCN gives each bone a sort of nervous system.

How the CCN actually senses mechanical loads remains unknown. There are many indications that both strain magnitude and strain rate are particularly important stimuli, but these strain parameters have the potential to stimulate bone responses in many ways (Pavalko et al., 2003; Martin, 2000). One well-studied hypothesis is that osteocytes sense shear stresses (Weinbaum et al., 1994). Osteocyte canaliculi are filled with fluid that is displaced every time a bone deforms, and the cells appear to be quite sensitive to pressure changes from these flows, inducing prostaglandin and nitric oxide (NO) production, and triggering communication at gap junctions (Cowin et al., 1995; Bakker et al., 2001, 2003). Characterization and study of the fluid flow within bone comprise a new frontier in bone research. For example, in vitro studies of osteoblasts showed that steady or pulsatile fluid flow produces greater response (measured as Ca^{2+} ion concentration in the cells) than oscillating flow (Jacobs et al., 1998). Likewise, the three-dimensional architecture and dimensions of canaliculi and lacunae have important implications for the distance that nutrients can be transported to osteocytes from Haversian canals and across which biochemical signals can act (Knothe Tate, 2003; Mishra and Knothe Tate, 2003).

Within the context of fluid flow within bone, strain appears to be an especially important stimulus that acts upon osteocytes in the CCN (Turner et al., 1995a). Strains measured in the body range from 0 (i.e., static loads) to >60 Hz; skeletal muscles contract at much higher frequencies (15–60 Hz) than most organismal activities such as the strains produced by walking (McMahon, 1984). This difference in rates raises the possibility that rapid oscillations associated with muscle contractions may provide a key signal to osteocytes. However, experiments on active and tranquilized dogs showed that high-frequency (15–30 Hz) strains generated only about 4% of the magnitude deformation of strains of 0–15 Hz, and thus were unlikely to have a major impact on bone deformation or fluid flow in the CCN (Turner et al., 1995b).

Another possibility is that osteocytes and osteoblasts sense strain in their plasma membranes, which contain stretch-activated ion channels (Guggino et al., 1989; Davidson et al., 1996) that permit Ca^{2+} flux, potentially initiating other intracellular responses. Yet another hypothesis, which is receiving increased attention, is that strain-induced fluid flow within bone matrix generates small changes in electrical charge (strain-generated potentials, SGPs; Cowin and Moss, 2001). In addition, Dodd et al. (1999) suggested that the interstitial fluid flow generated by loading is crucial for generating rapid diffusion of oxygen and nutrients to osteocytes. According to their research, even 24 hr of unloading can lead to a substantial increase in osteocyte hypoxia and, ultimately, osteocyte apoptosis. Because osteocyte death may initiate Haversian remodeling, the hypothesis of Dodd et al. (1999) provides an intriguing mechanism that may explain why the lack of loading leads to endocortical resorption. Finally, it is likely that osteoblasts lining a bone in the periosteum also act as mechanoreceptors, perhaps via processes known as primary cilia that were shown to function as mechanoreceptors in kidneys and other cells (Whitfield, 2003).

One of the few certainties about mechanotransduction is that it likely occurs by means of more than one stimulus and through multiple mechanisms. Regardless of these mechanisms, the next step is for the signals to be transmitted via the CCN to cells that can initiate a response (if any). Martin (2000) proposed a unified theory of bone remodeling in which he argued that bone lining cells will generally trigger remodeling unless inhibited by a signal from osteocytes in the CCN. The inhibitory signal (e.g., NO and prostaglandin) is produced by oseteocytes in response to mechanical stimulation. Martin (2000) argued that either diminished loading or microcracks (by physically damaging the network or by slowing fluid flow; Mori and Burr, 1993; Martin et al., 1998; Martin, 2000) can diminish the inhibitory effect, allowing the lining cells to activate osteoclasts and commence Haversian remodeling.

Pavalko et al. (2003) proposed a similar, but biochemically more specific, model for how mechanical strains utlimately lead to changes in gene expression in bone cells. The key innovation in the hypothesis by Pavalko et al. (2003) is the proposal that mechanical deformations eventually lead to the formation of multiprotein complexes that transfer biochemical signals from the extracellular matrix into the cells and then into the nucleus, and finally activate regulatory regions on the desired genes that need to be transcribed. Pavalko et al. (2003, p. 104) named these multiprotein complexes the "mechanosome" by which "bending bones ultimately bends genes."

Cell-to-cell signaling within the CCN can occur via SGPs and/or transmissions across gap junctions, although it should be noted that the latter occur at frequencies too slow to keep up with typical strain rates associated with muscles (McMahon, 1984). Various untested or partially tested models (e.g., Zhang et al., 1998) were formulated to predict how these signals may be integrated and amplified. In addition, how this system works as an ensemble is unclear. Presumably, the CCN functions somewhat analogously to an integrated neural network in which information on stimuli from receptor cells is weighted and/or summed by cells within the network, and then further transmitted to other skeleto-

genic cells if some threshold value is exceeded (Cowin and Moss, 2001; Turner et al., 2002). Moreover, because canalicular gap junctions are bidirectional, it is likely that the system permits mechanisms of feedback whose nature remains entirely hypothetical.

Much of the data used to formulate models of how osteocytes respond to stresses derive from in vitro studies, which should be interpreted with caution. Various kinds of bone cells may respond in different ways to stimuli in vitro once they have been removed from contact with other cell types in the CCN and other biochemical substances present in the extracellular matrix. For example, the strains that a living bone must experience for its cells to produce an osteogenic response are much smaller than the strains that bone cells in culture must experience to elicit the same response: 0.04–0.3% deformation in vivo vs. 1–10% deformation in vitro (You et al., 2001). The disparity may be due to amplification of fluid shear strains by the precellular matrix in vivo (You et al., 2001), but the fact remains that bone cells in vitro do not behave like cells in vivo in a variety of other ways. Thus, although in vitro studies can provide crucial, experimental insights into how cells work, generalizations from such studies must be viewed with caution until they are validated in vivo.

Another caveat to consider is that although the same basic mechanical, biochemical, and cellular mechanisms most likely operate similarly in humans as in mice, rats, dogs, or other domestic livestock, those mechanisms may differ subtly between species so that the concentrations of biomolecules needed to elicit responses may be different or the molecules may affect tissues in slightly different ways. As with in vitro studies of cellular function, experimental validation is needed. Animal models provide the best available means of conducting rigorous, in vivo experimental studies of the effects of mechanical loads on cells on tissues, but confirmatory results from humans are useful in making confident generalizations from animal models to humans.

How do applied loads induce osteogenic responses?

Once a bone experiences some type of strain that is sufficient to be sensed and transduced, the next step in any bone response to mechanical loading is the activation of osteogenic cells to elicit one of four potential outcomes. The first outcome is no response (quiescence), either because the signal was not sufficient (e.g., below a threshold value) or because the response was inhibited (a key point, as we shall see below in the context of aging). The second outcome is that osteoblasts are recruited in the periosteum or endosteum to grow new bone (hereafter termed "modeling"). The third outcome is resorption, in which osteoclasts are recruited to resorb bone along a surface, a process called "resorptive modeling" or "modeling in a resorptive mode." The final outcome is bone turnover, also known as Haversian remodeling in cortical bone, which occurs through a coordinated, sequential activation of osteoclasts and osteoblasts, known as a bone metabolic unit (BMU) (Martin and Burr, 1989). In a BMU, osteoclasts first resorb old bone in a tunnel-like fashion, and then osteoblasts deposit circumferential lamellae of bone within the tunnel around a vascular channel, forming a secondary osteon (Haversian system). Thus, before discussing what determines these outcomes and how they are affected by senescence, it is useful to review the basic physiology and regulation of osteoblasts and osteoclasts.

Osteoblasts

Osteoblasts are a general class of mesenchymal cells that not only form bone by synthesizing collagen matrix and then secreting calcium-phosphate mineral, but also include osteocytes and bone-lining cells that regulate other metabolic and possibly sensory functions of bone. Osteocytes and bone-lining cells may be different stages along a continuum of osteoblastic cell-line differention, although each of these cell types has its own set of biochemical markers than can distinguish them from osteoblasts. The recent establishment and study in vitro of osteocyte-like cell linages facilitated the study of osteocytes and their biochemical responses to strain and chemical signals (Bonewald, 1999; Cherian et al., 2003; Zhao et al., 2002; Cheng et al., 2001; Kato et al., 2001). Osteoblasts originate from mesenchymal precursor cells in a variety of tissues, including the periosteum and endosteum. Given its complexity, it is not surprising that osteoblast regulation is only very poorly understood (Ducy et al., 2000). Differentiation is most dominantly controlled by a key transcription factor, *Cbfa1* (core-binding factor-α1, also known as *Runx2*; Harada and Rodan, 2003), which in turn is regulated largely by the bone morphogenetic proteins (BMPs; see below). Knockout mice for *Cbfa1* lack any bone in their skeletons (Otto et al., 1997), and mutations in the *Cbfa1* gene in humans are associated with a wide range of osteogenic disorders such as cleidocranial dysplasia (Lee et al., 1997; Mundlos et al., 1997). *Cbfa1* is also involved in regulating osteoblast activity, specifically the rate at which osteoblasts secrete bone matrix (Ducy et al., 1999). The Indian hedgehog gene, *Ihh*, was also shown to regulate certain aspects of osteoblast function in endochondral growth, both in growth plates and in the periosteal collar (Olsen et al., 2000).

Quite a few growth factors affect osteoblast function, many of which are known to regulate *Cbfa1*. These factors primarily belong to five different families: the BMPs (particularly, BMP-2, -3, -4, and -7); the fibroblast growth factors (FGF-1 and -2); the insulin-like growth factors (IGF-1, IGF-2, and GFBP 3/5); transforming growth factor-β (TGF-β-1, and -2); and the platelet-derived growth factors (PDGF-AA, -AB, and -BB) (reviewed in Jee, 2001; Majeska,

2001). Most of these factors have multiple roles; indeed, BMPs are capable of activating the entire cascade of bone formation when exogenously introduced (Karsenty, 1998). For reviews of these factors, see Karsenty (2003), J.R. Lieberman et al. (2002), S.N. Khan et al. (2000), and Barnes et al. (1999). Osteoblasts, of course, are also regulated by the endocrine system, including parathyroid hormone (PTH); $1,25(OH)_2$ vitamin D; calcitonin; and the sex steroids. These hormones have quite varied roles, but estrogen is particularly important because of its role in osteoporosis (R.T. Turner et al., 1994; Westerlind et al., 1997). At normal doses, estrogen upregulates osteoblast (and chondroblast) activity; estrogen receptors in mature osteocytes as well as osteoblasts are also critical in regulating mechanotransduction (Lee et al., 2003). Estrogen acts on osteoblasts via two different receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ), which have very different effects (R.T. Turner et al., 1994). ERα plays a crucial role in mechanotransduction and osteogenic responses to strain. A number of ERα polymorphisms exist and are associated with significant variations in bone mineral density in juvenile and adult humans (Long et al., 2004; Boot et al., 2004). ERβ acts to protect and maintain bone mass, but in a complex way. A particularly high number of ERβ receptors are found in cells in cancellous bone (Bord et al., 2001) and in osteoclasts (Braidman et al., 2001). ERβ polymorphisms are associated with substantial variations in bone mineral density in postmenopausal women (Scariano et al., 2004). However, a study of $ER\beta^{-/-}$ knockout mice found no difference between male $ER\beta^{-/-}$ mice and wild-type males or between prepubertal female knockout and wild-type mice, but a substantially *increased* bone mineral content in adult $ER\beta^{-/-}$ females relative to wild-type females (Windahl et al., 1999). The increased bone mineral content in adult $ER\beta^{-/-}$ females was due to increased cortical bone thickness rather than trabecular density. The results suggest that functional ERβ receptors act to slow the rate of subperiosteal growth in female mice during adolescence (Windahl et al., 1999). These results were recently supported in humans by the results of a 15-year longitudinal study of bone loss in the distal radius of 108 perimenopausal women, which found an acceleration in the rate of endosteal resorption and (contrary to expectations) subperiosteal deposition following menopause (Ahlborg et al., 2003). The increase in the rate of subperiosteal bone deposition supports the hypothesis that ERβ receptors might be slowing this process in women in the presence of a sufficient concentration of estrogen.

Other hormones also exert influences on bone independent of or in tandem with estrogen. PTH and $1,25(OH)_2$ vitamin D_3 are essentially mineral-building or mineral-preserving hormones that stimulate osteoclasts and inhibit osteoblasts, the latter through inhibiting *Cbfa1* (*Runx2*) expression (Ducy et al., 1999). However, the actions of PTH are complicated and sometimes paradoxical; intermittent injections of PTH can also stimulate osteoblasts to produce bone (Majeska, 2001; Harada and Rodan, 2001). PTH also modulates the response of osteoblast-like cells in vitro, lowering the threshold needed to stimulate cellular response to bending-induced fluid flow (Ryder and Duncan, 2000). Calcitonin, a mineral-building hormone secreted by the thyroid, upregulates osteoblasts and downregulates osteoclasts. Leptin, a hormone produced by adipose tissue, may also affect osteoblasts via the hypothalamus through a complex pathway (Harada and Rodan, 2003). Leptin may play another important role with respect to bone by stimulating mesenchymal stem cells to commit to specific pathways of differetiation. High concentrations of leptin induce stromal cells to become osteoblasts and inhibit the differentiation of adipocytes (Thomas et al., 1999). Leptin may also have other effects on bone that may change during aging, and which differ slightly between the sexes (Thomas and Burguera, 2002).

How mechanotransduction specifically influences osteoblast regulation at the cellular level is very poorly known, particularly in relation to the above growth factors. In vitro experiments demonstrate that preosteoblasts play a crucial role in shaping bony responses to mechanical stresses, and that their responses to those stresses vary with their state of differentiation into mature osteoblasts (Weyts et al., 2003). Within minutes of in vivo loading, osteocytes exhibit an increase in uptake of tritiated uridium and have increased levels of glucose-6-phosphate dehydrogenase (G6PD) in proportion to the magnitude of strain (Skerry et al., 1989). In addition, pulsating fluid flow and in vivo strain increase levels of intracellular Ca^{2+} and protein kinase C in osteoblasts and osteocytes, which in turn stimulate the release of nitric oxide (NO) and prostaglandins (especially PGE_2) (C. Turner et al., 1994, 2002; C. Turner, 1998b, 1999; Hsieh et al., 1999; Pavalko et al., 1998, 2003; Owan et al., 1997; Burger and Klein-Nulen, 1999; Burr et al., 2002). Both NO and PGE_2 are potent anabolic regulators of bone growth. PGE_2 promotes recruitment of osteoblast precursor cells in mesenchyme, and stimulates both osteoblast proliferation and activity, as measured by alkaline phosphatase and collagen synthesis (Cui et al., 2001; Tang et al., 1997). In vivo inhibition of PGE_2 inhibits any skeletal response to mechanical strain (Forwood, 1996; Chow et al., 1998). NO also appears to be a key mediator of osteoblast activity, but its mechanism of action is less well-understood (reviewed in Ehrlich and Lanyon, 2002).

In short, although strain and fluid flow clearly trigger a cascade of cellular responses in osteoblasts, we know more about the effects of these stimuli than the mechanisms by which they are transduced and mediated intracellularly. For the purposes of this review, it is important to keep in mind that the rate of loading (the rate at which stress is increased and then released) appears to be particularly crucial in

eliciting a response from bone cells, and that high loading rates are more potent stimulators of new bone formation (Hsieh and Turner, 2001). In an extreme case, Rubin et al. (2001, 2002) found that rapid oscillations (hence high strain rates) of tiny strain magnitudes were able to produce bone modeling in turkeys and sheep (albeit only in cancellous bone). Duration and spacing of loading events are also important, as bone cells (studied in rats) evidently become quickly saturated after approximately 30–36 cycles of loading (Robling et al., 2001) and can show a significant osteogenic response even after five cycles (Umemura et al., 1997). Eight hours of recovery time are then required to restore the full responsiveness of cells (Robling et al., 2001). As a result, intermittent bouts of loading elicit a greater response than a single bout of loading, with all other variables held constant (Robling et al., 2000, 2001, 2002).

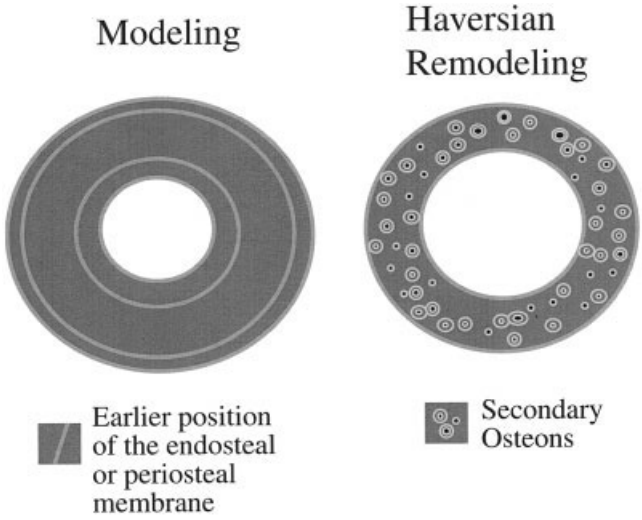

Fig. 5. Schematic representation of modeling and Haversian remodeling in cross-section of a long bone.

Osteoclasts

The other major cells to consider here are osteoclasts, which are large (up to 100 μm in diameter) multinucleated cells whose function is to resorb bone. Each osteoclast derives from 10–20 mononuclear phagocytes in hematopoietic marrow (Teitelbaum et al., 1996; Boyle et al., 2003), and thus is a part of the macrophage cell lineage. When activated, osteoclasts form a ruffled surface that adheres to a bone surface, creating a seal necessary for resorption to take place. Resorption occurs from the combined effect of releasing H_2CO_3 to decrease local pH to approximately 3.5, and through secretion of various proteolytic enzymes that digest collagen.

Thus far, at least 24 genes are known to have a positive or negative effect on osteoclastogenesis or osteoclast function (Boyle et al., 2003), including several transcription factors that regulate osteoclast differentiation such as Pu.1, *c-fos*, and NF-κB (reviewed in Karsenty, 1999). Mutations of these genes typically result in abnormally dense bone (osteopetrosis) (McLean and Olsen, 2001; Van Wesenbeeck et al., 2002; Boyle et al., 2003) or accelerated osteoclastic activity that produces osteopenia or osteoporosis (Takeshita et al., 2002; Bucay et al., 1998). Osteoclast transcription, in turn, is regulated by a number of secreted molecules, including two pairs of effectors that are synthesized (crucially) by osteoblasts as well as other cells: 1) osteoprotegerin ligand (OPG-L) and 2) osteoprotegerin (OPG) (Jee, 2001; Boyle et al., 2003). To confuse matters, OPG and its ligand are also known as RANK and RANKL, respectively (Yasuda et al., 1998). OPG-L/RANKL stimulates the differentation and activity of osteocytes, and inhibits osteoclast apoptosis (cell death). In contrast, OPG/RANK inhibits the differentiation and activation of osteoclast precursor cells, downregulates osteoclast activity in mature osteoclasts, and induces apopotosis. Consequently, the ratio of OPG-L/RANKL to OPG/RANK may be an important determinant of bone remodeling activity (Khosla, 2001). Numerous other signaling factors and hormones also influence osteoclast regulation. Stimulators include PTH, $1,25(OH)_2$ vitamin D_3, thyroid hormone, glucocorticoids, IGF-I, and BMP-2 and -4; inhibitors include calcitonin, nitric oxide (NO), gonadal steroids, and interleukin-1 and -6 (Jee, 2001; Boyle et al., 2003).

How exactly a strain stimulus leads to stimulation or inhibition of osteoclasts is not yet known, but it seems most likely that the regulation primarily occurs via soluble signals released by osteoblasts, particularly lining cells. Osteoclasts have several membrane receptors, known as integrins, which interact with extracellular proteins including collagen, opsteopontin, and bone sialoprotein (Nakamura et al., 2003). These then modify the ratio of OPG-L/RANKL to OPG/RANK, which then upregulates or downregulates the activity of osteoclasts, leading to a range of outcomes from quiescence to varying rates of resorption. Osteoblasts thus appear to play a central integrative role in regulating osteoclasts in response to mechanical loading.

Cellular coordination of ostebolasts and osteoclasts

Regardless of the molecular mechanisms by which cells (mostly in the osteoblast-lineage) regulate osteoblasts and osteoclasts, the potential outcomes of the above pathways are either quiescence, modeling (periosteal and/or endosteal deposition or resorption), or Haversian remodeling (bone turnover) (Fig. 5). Of these, modeling is probably the best understood in terms of its mechanical function. Depositional modeling makes bones stronger in two ways. First, by augmenting cross-sectional area, the addition of bone mass helps to counteract compressive forces by distributing them over larger areas (reducing stress). Second, modeling increases resistance to bending and twisting by augmenting second mo-

ments of area (SMA, I) available to resist the moments that account for a high proportion of midshaft strains (Bertram and Biewener, 1988). Adding bone subperiosteally is particularly useful in this regard because bone added further away from an axis of bending resists bending much more effectively than the same amount of bone added near the axis (Martin et al., 1998). By adding bone, especially adding bone subperiosteally, modeling reduces the strain a given moment generates (Pauwels, 1974; Wainright et al., 1976; Alexander, 1981; Currey and Alexander, 1985). There is abundant evidence that modeling increases second moments of area in response to loading in juveniles, mostly through increases in periosteal apposition (Chamay and Tchantz, 1972; Goodship et al., 1979; Lanyon, 1982; Lanyon and Rubin, 1984; Rubin and Lanyon, 1984, 1985; Biewener et al., 1986; Raab et al., 1991; Lieberman, 1996; Bass et al., 1998; Ruff et al., 1994; Lieberman and Pearson, 2001; Lieberman et al., 2003, 2004a), and to a lesser extent through inhibition of endosteal resorption (Woo et al., 1981).

The other potentially major outcome of increased mechanical loading is Haversian remodeling via activation and coordination of osteoclasts and osteoblasts within BMUs (see above). The function of Haversian remodeling is not entirely understood (reviewed in Martin et al., 1998; Currey, 2002; Parfitt, 2002). One hypothesis is that it prevents or repairs fatigue damage caused by high strain magnitudes and/or frequencies. Repair of fatigue damage is called targeted remodeling; Haversian remodeling that occurs in random locations with respect to concentrations of microcracks is termed nontargeted or stochastic remodeling (Burr, 2002). One study of the association between Haversian remodeling and microcracks in dog radii suggests that approximately 30% of Haversian remodeling is directed; 70% is stochastic (Burr, 2002; Mashiba et al., 2001). Given that not all microcracks that might initiate Haversian remodeling events will be visible in a thin section, Martin (2002) calculated that it is possible that *all* Haversian remodeling events are initiated by microcracks, but this hypothesis needs to be tested experimentally.

However Haversian remodeling is initiated, Haversian bone is weaker in vitro than young primary osteonal bone (Currey, 1959; Carter and Hayes, 1977a,b; Vincentelli and Grigorov, 1985), but is stronger than old primary bone that has accumulated microcrack damage (Schaffler et al., 1989, 1990). Haversian systems may also halt microfracture propagation (Currey, 2002), and can strengthen bone by reorienting more collagen along axes of tension (Martin and Burr, 1982; Riggs et al., 1993a,b). Not surprisingly, many studies showed that loading elevates Haversian remodeling rates, especially in older regions of long bones that have presumably accumulated the most damage (Heřt et al., 1972; Frost, 1973; Bouvier and Hylander, 1981, 1996; Churches and Howlett, 1981; Rubin and Lanyon, 1984, 1985; Schaffler and Burr, 1988; Burr et al., 1985; Mori and Burr, 1993; Goodship and Cunningham, 2001; Lieberman and Pearson, 2001; Lees et al., 2002; Lieberman et al., 2003). One possible explanation for this observation is that osteocyte apoptosis may inititiate Haversian remodeling (Burr, 2002; Noble et al., 1997). The accumulation of microcracks from loading may also lead to osteocyte apoptosis (Verborgt et al., 2000), in essence mimicking this effect of aging.

Although old bone almost certainly has accumulated microdamage, there are other reasons why it becomes progressively weaker than young bone. Prominent among these reasons is that both collagen and hydroxyapatite crystals undergo chemical alterations that diminish their ability to resist tensile and compressive loads, respectively (Banse et al., 2002; Zioupos, 2001; Bailey et al, 1999; Bailey and Knott, 1999; Akkus et al., 2004).

Finally, less-than-normal or otherwise low levels of loading can lead either to no response (quiescence) or to resorption through activation of osteoclasts. Typically, reduced loading occurs somewhat systemically from long periods of bedrest or space flight. For example, astronauts often lose up to 7% of skeletal mass, especially in the distal leg, even following countermeasures such as resistance-generating exercise (Baldwin et al., 1996; Collet et al., 1997). The one region of the skeleton that does not appear to lose bone is the cranial vault, where osteoclasts do not differentiate despite a low strain environment (Lieberman, 1996; Goodship and Cunningham, 2001). In addition, numerous experiments showed that localized resorption occurs following changes in the musculoskeletal system that reduce mechanical strains in a single region, such as amputation or tooth loss. For example, limb immobilization causes increases in endosteal resorption that can often be rescued in juveniles but not adults (Uhthoff and Jaworski, 1978; Jaworski et al., 1980; Skerry and Lanyon, 1995), and loss of dental function can reduce alveolar crest size by as much as 50% (Carlsson and Persson, 1967; Israel, 1973; Sugimura et al., 1984).

Why low levels of loading lead to resorption is controversial. One long-standing hypothesis that is embedded within many formulations of Wolff's "law" is that such losses return a bone to its original genetically predetermined shape. In a classic experiment, Chalmers and Ray (1962) grew an unloaded rat femur within a spleen, causing the bone to have thinner cortices; less organized trabeculae; a short, wide ("unwaisted") femoral neck; and no diaphyseal curvature. One problem with this hypothesis, however, is that given the complexity of the epigenetic pathways by which bones interact with muscles, other tissues, and mechanical forces, there is probably no such thing as a purely genetically determined shape. Rather, both genes and environment (including the strain history of the bone and other extrinsic influences such as health and nutrition)

combine to produce a bone's morphology over long periods of time. Another hypothesis is that localized resorption is a mechanism to rid the skeleton of excess mass. Finally, and most likely, extended periods of net skeletal resorption may be primarily a pathological consequence of a lack of epigenetic stimulation (e.g., Dodd et al., 1999), which has not been acted upon (or remediated) by natural selection because of the rarity of prolonged shortfalls of epigenetic stimulation in nonhibernating organisms. Indeed, the activities of osteoclasts and osteoblasts are usually coordinated (Martin et al., 1998), and the pathways of bone regulation are sufficiently complex that inhibition of resorption (resorption in general or of the prolonged net resorption in later decades of life that leads to osteoporosis) may be difficult to evolve because it also inhibits other more important functions that involve the coordination of osteoclasts and osteoblasts and thus have a negative impact on fitness. An extreme example of this problem is cherubism, a disorder characterized by intense bone resorption in the maxillae and the lower jaw coupled with deposition of cellular fibrous tissue (Ueki et al., 2001; Tiziani et al., 1999).

EFFECTS OF AGING ON BONE CELLS

It has long been known that mechanical loading primarily influences the juvenile skeleton, when most of the skeleton's growth in size occurs (Bertram and Swartz, 1991). Nevertheless, the bones of adult animals have also often been assumed to be very responsive to changes in mechanical loading, a premise that is only partially true. As reviewed in much more detail below, it has become clear that mechanical loading produces greatly decreased modeling and remodeling responses in the cortical bone of skeletally mature animals (Lieberman et al., 2003). Before examining the evidence for the effects of aging on skeletal modeling and remodeling, we review a few aspects of osteoblast senescence.

In adults, osteoporosis results from a chronic excess of resorption of bone by osteoclasts in comparison to the amount of bone deposition by osteoblasts (Teitelbaum et al., 1996). Changes in hormone levels (especially estrogen in females and testosterone in males) appear to be the prime cause of the imbalance between resorption and deposition, but the senescence of osteoblasts and of osteoprogenitor cells also plays a role. In one important study, Nishida et al. (1999) investigated senescence in osteoprogenitor cell populations in the iliac bone marrow of 49 females between 4–88 years of age. Bone marrow cells cultured in vitro formed colony-forming units-fibroblastic (CFU-Fs) that can express osteoblastic phenotypes as gauged by alkaline phosphatase (ALP) activity, production of mRNA for osteocalcin (OLC) and parathyroid hormone-receptor (PTH-R), and formation of calcified nodules in the culture medium. Nishida et al. (1999) found a marked decline in the number of ALP-expressing CFUs after age 10 (Fig. 6), and interpreted this result to indicate an age-related decline in the number of osteoprogenitor cells that can be recruited to form osteoblasts.

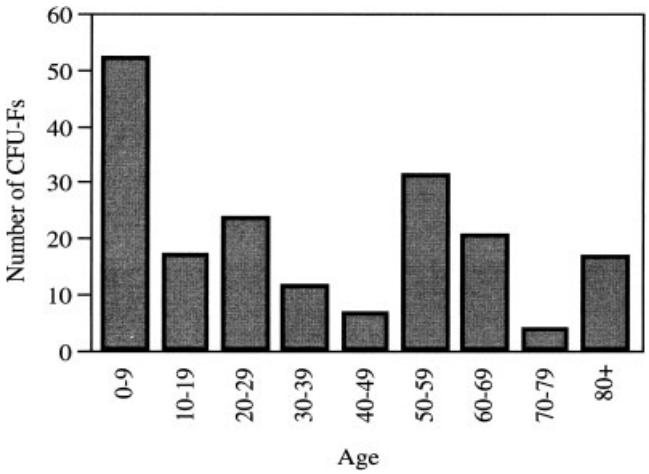

Fig. 6. Decline in colony-forming units ((CFU-Fs, recruited from osteoprogenitor cells) with age (data from Nishida et al., 1999).

The results of Nishida et al. (1999) corroborated other studies' findings that bone marrow cells from older individuals have a diminished capacity to differentiate into osteoblasts and produce new bone (Tsuji et al., 1990; Liang et al., 1992; Quarto et al., 1995; Bergman et al., 1996; Majors et al., 1997; Inoue et al., 1997; Kohrt, 2001). Muschler et al. (2001) reported a similar age-related decline in osteoprogenitor cells in a smaller sample of people (31 men, 26 women), but also found that the age-related decline was statistically significant in women but not in men. However, in an in vitro experiment, Justesen et al. (2002) reported no age-related decline in marrow cells' ability to differentiate into osteoblasts. Bone marrow cells cultured in vitro also have a fivefold decline in osteoprotegerin production (Makhluf et al., 2000), a change that may increase the ability of the stromal cells to produce osteoclasts. The density of lacunae for osteocytes decreases with age in human cortical bone, and as the number of osteocytes decrease, microcracks accumulate and gradually weaken the bone (Vashishth et al., 2000) and may also lead to osteocyte apoptosis (Verborgt et al., 2000). In dogs, the density of lacunae and the number of new osteons produced decline exponentially with age (Frank et al., 2002). The dogs' cortical bone also accumulated microcracks with age. These changes combine to make the bone of older individuals more susceptible to fractures and less responsive to sensing stimuli from their mechanical environment.

Aging is also associated with decreases in the ability of surviving cells to respond to a variety of molecular and mechanical stimuli. Tanaka and Liang (1996) found that old osteoprogenitor cells from rats required a dose of IGF-I two orders of magnitude higher than younger cells in order to elicit either mitogenic or proliferative responses.

The concentration of TGF-β and IGF-I decreases with age in the human femoral cortex; the decline in IGF-I is more marked ($r = -0.43$), while the diminution of TGF-β is less substantial ($r = 0.28$ for the inverse of TGF-β vs. age) (Nicolas et al. 1994). Results were similar in men and women, and the decline in IGF-I and TGF-β from 20–60 years amounted to 60% and 25%, respectively (Nicolas et al., 1994). Bätge et al. (2000) studied the senescence of receptors I, II, and III for TGF-β in osteoblasts from samples of human femoral cortex in 19 patients between 2–83 years of age. The patients' osteoblasts showed no significant change in the ability of the cells to differentiate or proliferate with age, but the number of TGF-β receptors per cell increased significantly with age (Bätge et al., 2000). The increase in TGF-β receptors in older cells appeared to be independent of IGF-I concentrations (Bätge et al., 2000). Similarly, Ankrom et al. (1998) reported that the number of estrogen receptor-α molecules per osteoblast doubled between their sample of younger and older (>50 years) women. The increased number of TGF-β and estrogen-α receptors on old bone cells appears unable to compensate for other age-related changes.

Osteoblasts in older rats are also less sensitive to mechanical signals from flow-induced calcium ion oscillations than those of younger individuals (Donahue et al., 2001). In a comparison between tibial growth in response to applied mechanical loading in adult (9-month-old) and aged (19-month-old) rats, Turner et al. (1995c) reported that the older rats' tibias responded by growing less frequently and less exuberantly in response to applied loads. The older rats' cells appeared to have increased thresholds for responding to strain. For the endosteal surface of the tibia, Turner et al. (1995c) quantified the threshold for generating an osteogenic response as 1,700 microstrains (με) vs. 1,050 με for the old and young animals, respectively. Stanford et al. (2000) reported similar results from a study of bone cells of humans over 60 years of age in which cells were loaded at 10,000 cycles of 1 Hz or 10,000 cycles at 20 Hz loading at 1,000 microstrains per cycle, or not loaded at all. The difference in osteogenic response between the samples indicates that cells cultured from older humans do not respond to strains that elicit a response in younger indidividuals.

The question of why osteoblasts and osteoprogenitor cell lines senesce is very difficult to answer. Most likely, the senescence of osteoprogenitor cells, an evolvable aspect of life history, results from similar mechanisms to those responsible for senescense in other tissues (reviewed in Rose, 1991; Hill, 1993). Currently popular hypotheses for aging include the gradual accumulation of damage from oxidative stress, programmed cell death, epigenetic modifications of chromosomes including methylation or other alterations that cells cannot reverse, telomere shortening, and the production of gene products that harm cells (Weinert and Timiras, 2003; Ben-Porath and Weinberg, 2004; Mathieu et al., 2004). The search for the genes and epigenetic effects responsible for slowing or speeding the process of aging is currently very active and is producing exciting results (e.g., Gonos et al., 1998; Holzenberger et al., 2003; Collins and Sedivy, 2003; Söti et al., 2003).

MACROSCOPIC EFFECTS OF AGE AND EXERCISE ON LONG BONE CROSS-SECTIONS AND BONE DENSITY

Here we turn to the macroscopic effects of bone cell responses to mechanical loading and how these responses change during ontogeny. After reviewing several general models for the effects of age on cortical bone modeling and remodeling, we examine several problems of particular interest to biological anthropologists: modeling and cross-sectional geometry of bone shafts; bone turnover (Haversian remodeling); and bone mineral density. Although the literature is replete with hundreds of studies that examined various aspects of bone responses to loading both in vitro and in vivo, we focus primarily on the comparative human data and the most relevant animal studies that help test the most important models. For the most part, these data support the general conclusion that cortical bone responses to loading occur primarily in juveniles. Unfortunately, there is little definitive evidence that can be used to support or reject most models conclusively because of the complexity of the system, and because few studies have looked at more than one bone and at more than one ontogenetic stage. In addition, few experiments examined multiple levels of response to loading from the cellular to the gross structural levels.

Models for the effects of age on bone modeling and remodeling

As with any complex phenomenon, models are crucial to making sense of the variable outcomes of the many factors and interactions discussed above that contribute to bone responses to loading in different parts of the skeleton and during different periods of ontogeny. Over the last few decades, several general descriptive models as well as a number of predictive equilibrium and optimization models were proposed to explain age-related variations in the occurrence and rate of modeling and (sometimes) Haversian remodeling in the skeleton in response to mechanical loading (Table 1). Therefore, before reviewing the human and animal data, it may be useful to summarize the major details and assumptions of these models.

Descriptive models

The first set of models for how age affects bone modeling is purely descriptive and not focused upon the issue of age-related changes in Haversian remodeling. Many clinical studies show that juvenile humans who exercise build considerably more bone

TABLE 1. Models of bone modeling and remodeling and their predictions with respect to age

	Effect of loading on modeling		Effect of loading on Haversian remodeling (HR)	
	Juveniles	Adults	Juveniles	Adults
I. Descriptive models				
Ruff et al. (1994)	Increases periosteal deposition, slows endosteal resorption.	Minor increase in periosteal deposition, but slows endosteal resorption or causes endosteal deposition.	No prediction	
II. Equilibrium models				
Dynamic strain similarity (Rubin and Lanyon, 1984); dynamic equilibrium (Biewener et al., 1986)*	Periosteal and endosteal modeling in both growth and adulthood to keep peak strains within a particular range. Juveniles and adults are similar, but equilibrium in adults is not continuously disrupted by somatic growth.		No prediction	
Mechanobiology hypothesis (Carter and Beaupré, 2001)	Growth = r_m (mechanical) + r_b (biological) components. Bones model to experience optimum strain levels. r_b is more important in growth and thus during juvenile period.		No prediction, but presumably HR is heavily influenced by r_b (biological component) and changes during life span.	
Mechanostat (Frost, 1987, 1990)[1]	High strains simulate deposition (modeling). Low strains stimulate resorptive modeling.		High strains inhibit HR. Low strains promote HR.	
III. Optimization models				
Trade-off model (Lieberman and Crompton, 1998)	Modeling varies by skeletal location. High strains produce modeling, but mostly in more proximal bones in a limb.[2]		HR varies by skeletal location. High strains induce HR, but more HR will occur in distal bones in a limb.[2]	

[1] These models apparently conceptualize physiological components of processes of growth and development as extrinsic to response that bone tissue displays in response to mechanical loading.
[2] Ontogenetic age alters strength of modeling and Haversian remodeling responses (Lieberman et al., 2003; Lieberman and Pearson, 2001).

than their nonexercising peers, resulting in higher peak bone mass as young adults. Because bone is progressively lost throughout later adulthood, a high peak bone mass in young adulthood reduces the risk of fractures associated with clinical osteoporosis later in life. Most of the studies summarized below indicate that exercise stimulates bone modeling (growth), most potently around puberty when hormonal levels surge (e.g., Bass et al., 1998, 2002; Haapasalo, 1998; Haapasalo et al., 1998; K. Khan et al., 1998, 2000; but see Kardinaal et al., 2000, Sundberg et al., 2001, 2002). As noted above, these data have important ramifications for interpreting Wolff's "law" (Bertram and Swartz, 1991), and recently some scholars incorporated ontogenetic age into their models of skeletal responses to mechanical loading. Of the various descriptive models for the ontogeny of long bone cross-sectional properties, that of Ruff et al. (1994) is the most influential, in large part because it provides a developmental and functional mechanism to explain the large body of evidence that Pleistocene hominids and preagricultural and other preindustrial modern humans tend to have long bones with very thick cortical bone, narrow medullary cavitities, and elevated bending strength (Weidenreich, 1941; Endo and Kimura 1970; Day, 1971; Trinkaus, 1976; Senut, 1985; Ben-Itzhak et al., 1988; Trinkaus and Ruff, 1989; Bennike and Bohr, 1990; Ruff et al., 1993).

The model by Ruff et al. (1994) (Fig. 7) is based on observations that juveniles typically deposit additional amounts of subperiosteal bone and slow the rate of endosteal resorption in response to strenuous mechanical loading; in contrast, adults who became active after a sedentary childhood tend to slow the rate of endosteal resorption, but are not able to add substantial amounts of new subperiosteal bone (Garn et al., 1967, 1969; Garn, 1972; Frisancho et al., 1970; Ericksen, 1976; Lazenby, 1990). The consequence of these different responses to similar stimuli is that mechanical loads prior to skeletal maturity will result in greater external dimensions of a long bone's shaft and narrower medullary cavities. However, mechanical loads during adulthood have little effect on the external dimensions of long bones diaphyses, but result in greater cross-sec-

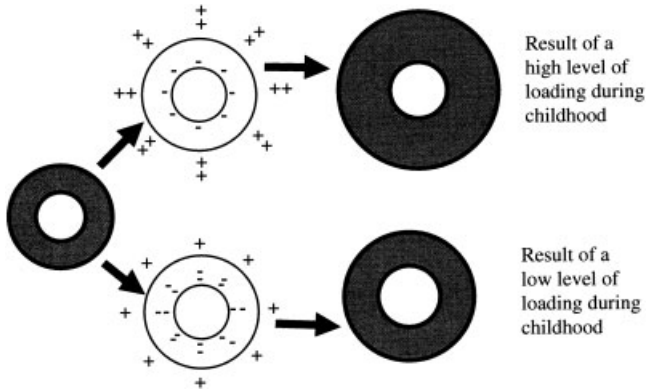

Fig. 7. Model by Ruff et al. (1994) for ontogenetic changes in bone modeling in response to loading.

tional areas from smaller medullary cavities. Biomechanically, the adult pattern of diaphyseal response is less efficient, because the contribution of a given unit of area to a cross-section increases bone strength in proportion to the square of its distance to the neutral axis (Wainright et al., 1976).

Equilibrium models

While descriptive models are useful for interpreting variations in cross-sectional geometry, predictive models are necessary to interpret variable responses among different regions of the skeleton and are crucial for tests of causal hypotheses about the potential relationship between form and function. Most predictive models are equilibrium models, which hypothesize that cortical bone's responses to external forces act to maintain a mechanically stable system, keeping bones within a certain range on the stress-strain curve (Fig. 3A). These models primarily focus on periosteal/endosteal growth (modeling) and resorption, and make few, if any, predictions about Haversian remodeling, which has much less of an effect on bone strength than modeling (see above). The best-known equilibrium models are the related hypotheses of dynamic strain similarity and dynamic equilibrium (Rubin and Lanyon, 1984; Biewener et al., 1986), which propose that bones alter their cross-sectional geometries during growth to keep peak strains at similar ranges and below some threshold. Evidence for dynamic strain similarity comes from several sources, most notably that peak strain magnitudes recorded on the surfaces of various bones in diverse taxa during vigorous activities (e.g., galloping, flying, or eating hard food) vary independently of body mass between a range of 2,100–3,200 $\mu\epsilon$ (Rubin and Lanyon, 1984; Rubin, 1984; Biewener, 1991). In addition, diaphyses in several species were shown to grow during ontogeny in a way that maintains constant magnitudes and orientations of in vivo strain at functionally equivalent sites despite increases in mass and length by 10-fold and 3-fold, respectively (Biewener et al., 1986; Biewener and Bertram, 1993; but see Main and Biewener, 2004).

One problem with dynamic strain similarity/equilibrium hypotheses is that they do not consider the effects of age following skeletal maturity. Carter and Beaupré (2001) described a comprehensive equilibrium model, the mechanobiological hypothesis (which predicts ontogenetically variable interactions between intrinsic growth and responses to loading on bone modeling). According to this model (van der Meulen et al., 1993; van der Meulen and Carter, 1995; Carter and Beaupré, 2001), bone growth can be decomposed into two portions, a "biological" component, r_b, and a "mechanobiological" component, r_m. The biological component of bone growth is thought to reflect mostly "intrinsic" growth as determined by genes and hormones. The influence of r_b is expected to follow the growth velocity curve from development in utero into adulthood, thus experiencing an exponential decline into midchildhood, followed by a modest increase during the adolescent growth spurt, and finally a decrease to close to zero as linear growth ceases. In contrast, the mechanobiological component of the rate of bone growth, r_m, incorporates the hypothesis that bones model to attain an optimum level of strain in response to expected mechanical loads (Bertram and Biewener, 1988; Rubin and Lanyon, 1984). As ontogeny progresses, r_m becomes increasingly important and is thought to vary in order to maintain daily stress stimulus at each skeletal location (the product of strain energy magnitudes and the number of loading cycles) within a "lazy zone" estimated to be approximately 30–70 MPa/day. Within the "lazy zone," increased or decreased levels of strain are predicted to stimulate little apposition or resorption. Above or below the "lazy zone," however, increased loads will trigger apposition in proportion to the total daily strain energy, while decreases in loading induce resorption in proportion to the shortfall in the daily-expected strain energy. Thus, according to the model, decreased or increased loading later in life will primarily affect the rates of periosteal deposition and endosteal resorption. Carter and Beaupré (2001) furthermore proposed that site- and age-specific modeling rates are expected to vary depending on the presence of precursor cells (i.e., osteoprogenitor cells, ability to recruit and mobilize osteoclasts).

A series of simulation studies demonstrated that the mechanobiology model does accurately predict general patterns of growth in the cross-sectional geometry of the femoral midshaft during the period from childhood into adulthood: as body mass increases, so does the bone cross-sectional area necessary to maintain a similar section modulus (Carter et al., 1996; van der Meulen et al., 1993, 1996; van der Meulen and Carter, 1995). Challenges to the mechanobiology model, however, arise from the fact that it cannot explain why bone at different locations in the skeleton maintains very dissimilar strain thresholds for initiating modeling (Turner et al., 2002), and from the observation that osteocytes and osteoblasts rapidly become saturated when exposed to loading cycles of a high enough strain rate and amplitude to trigger an osteogenic response, and then need up to 8 hr to recover (Robling et al., 2000, 2001, 2002).

Only one equilibrium model, the mechanostat hypothesis, explicitly considers Haversian remodeling in relation to modeling. According to various formulations of the mechanostat (Frost, 1986, 1987, 1990), loads that generate strains above some threshold on the stress-strain curve stimulate growth and inhibit Haversian remodeling, whereas loads that generate strains below some other threshold on the stress-strain curve inhibit growth and stimulate loss of bone, including Haversian remodeling. Between these thresholds is a predicted equilibrium range in which loading stimulates neither modeling nor remodeling. The mechanostat specifically predicts

high rates of Haversian remodeling in understrained bone, thereby increasing bone porosity and leading to decreased stiffness and strength. Experimental studies, however, provide only limited support for the mechanostat. While high strains often elicit modeling, and low strains elicit resorption (summary in Martin et al., 1998, p. 260–264), high strains frequently stimulate Haversian remodeling in cortical bone (Goodship and Cunningham, 2001; Lieberman et al., 2003). In addition, the mechanostat does not explain age-related changes in modeling and remodeling responses to loading (see below).

Optimization models

While equilibrium models explain many structural variations that apparently maintain bone stiffness and strength within some threshold range at particular sites, they make few explicit predictions to account for the substantial variation evident between bones, between ontogenetic stages, and between species in both strain magnitudes and osteogenic responses to loading. It is well-known that safety factors (the ratio of maximum load capacity to observed loads) vary considerably within the skeleton (Alexander, 1998; Currey, 2002), and habitual activities generate an enormous range of strain levels (Gross et al., 1992; Rubin and McLeod, 1996; Demes et al., 2001; Lieberman et al., 2004a). Peak strain magnitudes at many locations throughout the skeleton are often considerably lower than the range of peak values noted above ($\approx$3,200 $\mu\epsilon$), presumably because of variations in the nature of loading dynamics, cross-sectional geometry, and other behavioral and mechanical factors (e.g., in the skull: Hylander and Johnson, 1997; Ross, 2001). Consequently, most equilibrium models acknowledge that regions of the skeleton may differ in the equilibrium range in which higher than normal strains stimulate modeling, and lower than normal strains stimulate either resorption (Carter and Beaupré, 2001) and/or Haversian remodeling (Frost, 1990). Moreover, the lability of these responses to loading, and hence the equilibrium range, is known to change with age (Carter et al., 1996; van der Meulen et al., 1993, 1996; van der Meulen and Carter, 1995).

Optimization theory may therefore usefully predict how cortical bone in *different* regions of the skeleton modulates modeling vs. remodeling in response to loading. Specifically, Lieberman and Crompton (1998), Lieberman and Pearson (2001), and Lieberman et al. (2003) proposed that if bones optimize strength relative to the cost of adding mass, and if Haversian remodeling repairs or prevents microdamage, then proportions of modeling vs. Haversian remodeling responses to loading should vary at different skeletal locations and ages in relation to their costs and benefits. From a mechanical standpoint, the major benefit of modeling is to strengthen a bone by increasing the second moment of area, I, around the axes in which applied bending forces generate deformation. The major

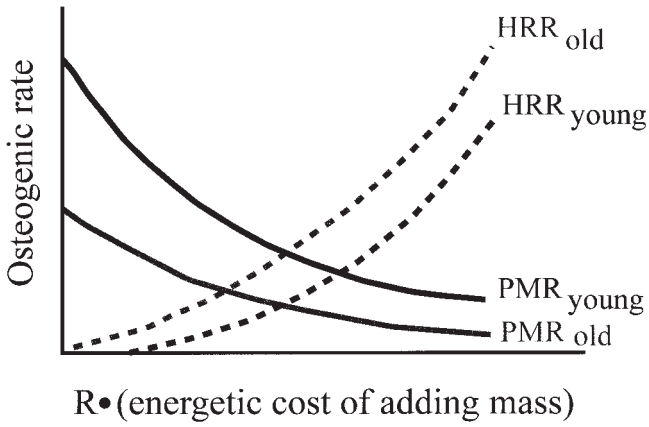

Fig. 8. Expectations from trade-off model by Lieberman and Crompton (1998) for modeling and Haversian remodeling in different skeletal sites within a limb (after Lieberman et al., 2003).

long-term cost of modeling varies, depending on skeletal location. In the skull or other complex regions, adding mass in many locations may result in structural imbalances that prevent normal function. In limbs, modeling requires additional energy to accelerate the added mass during locomotion. As stride frequency diverges from the natural oscillation frequency, the cost of accelerating the limb is roughly proportional to mR^2, where m is the mass of the limb, and R is the distance from the center of mass of the limb to the hip or shoulder joint (Hildebrand, 1985; Winter, 1990; Marsh et al., 2004). Thus the long-term energetic cost of adding bone mass is exponentially higher in distal vs. proximal elements, presumably accounting for the general phenomenon of limb tapering (Fig. 8).

The costs and benefits of Haversian remodeling are much less understood, but differ considerably from those of modeling. As noted above, proposed benefits of Haversian remodeling include replacing and thereby strengthening fatigue-damaged bone, increasing elasticity, halting microcrack propagation without adding mass or changing shape, and temporarily reducing bone mineralization, which increases its toughness and heterogeneity, both of which can halt crack propagation (Martin et al., 1998; Schaffler et al., 1990; Currey, 2002). But Haversian remodeling produces a transient increase in porosity and incurs higher long-term metabolic costs than modeling by leaving a bone insufficiently strong to resist further strain damage, requiring subsequent growth or remodeling (Martin, 1995). Consequently, using an optimization perspective (Fig. 8), Haversian remodeling responses to loading are predicted to be higher in biomechanically comparable more distal locations in a limb, where stresses and strains are higher, creating more microdamage and where the cost of modeling is high (Lieberman and Crompton, 1998; Lieberman et al., 2003).

The final component of the optimization model is how age affects rates of modeling and Haversian

remodeling. As noted above, as osteoprogenitor cells senesce, they decline in number and become less sensitive to many epigenetic stimuli, including those from mechanical loading (Muschler et al., 2001; Chan and Duque, 2002). In vitro and comparative studies indicate that in older individuals, osteoblasts are less responsive to strains than osteoblasts in growing individuals (Erdmann et al., 1999; Stanford et al., 2000; Donahue et al., 2001). In addition, comparative studies of the effects of exercise on the skeleton in different age groups show that mechanical loading stimulates periosteal growth mostly prior to skeletal maturity, and primarily acts to slow down the rate of bone loss in older individuals (e.g., Ruff et al., 1994; Bass et al., 1998; Wolff et al., 1999; Kohrt, 2001). Therefore, if modeling responses to strains decline as the skeleton ages (Fig. 8), then Haversian remodeling rates should increase in proportion to strain magnitude for all individuals, but the rate of increase should be higher for older animals if Haversian remodeling functions to repair or prevent fatigue damage (Lieberman et al., 2003).

Evidence for interactions between age and loading on Haversian remodeling

Experimental evidence shows that loading does stimulate Haversian remodeling, but in complex ways that change across the life span. The only equilibrium model that predicts patterns of Haversian remodeling is the mechanostat (Frost, 1987). In light of experimental findings, the mechanostat's predictions are clearly problematic, not only in terms of modeling (see above), but also in terms of Haversian remodeling. Although other studies that evaluated the mechanostat (e.g., Robling et al. (2002) found that equivalent magnitudes of applied stresses were more effective in producing modeling responses if the loads were separated into discrete bouts), Lieberman et al. (2003) offered probably the most direct test of the mechanostat as well as the trade-off model (Lieberman and Crompton, 1998).

To test these models, Lieberman and Crompton (1998) devised a set of experiments using male Dorset sheep (*Ovis aries*) of different ages. In each experiment, one group of sheep was trained to trot at a moderate pace 1 hr per day for 90 days, while a second group of control animals remained in their pens and did not exercise. Every 30 days during the experiment, a fluorescent bone-labeling dye (calcein, oxytetracycline, and xylenol orange) was administered via interperitoneal injection. The sheep were divided into three age groups: juveniles (n = 10, aged 40 days at the start of the experiment), subadults (n = 10, aged 265 days at the start), and young adults (n = 16, aged 415 days at the start). Initially, animals of different ages were studied because the investigation of age-related changes in the responses of bone is crucial for osteoporosis research and treatment interventions. Earlier experiments also showed that juvenile pigs showed remarkable changes in facial architecture and thickening of their cranial bones, and changes in response to variation in food consistency and exercise regimes (Corruccini et al., 1992; Lieberman 1996); similarly impressive changes were noted in pig postcranial skeletons in exercise interventions (Woo et al., 1981). In contrast, old adult humans tend to show only minor gains in bone mass in response to exercise interventions intended to build bone mass or slow its loss (Vuori, 1996; Turner, 1998a; Karlsson, 2001).

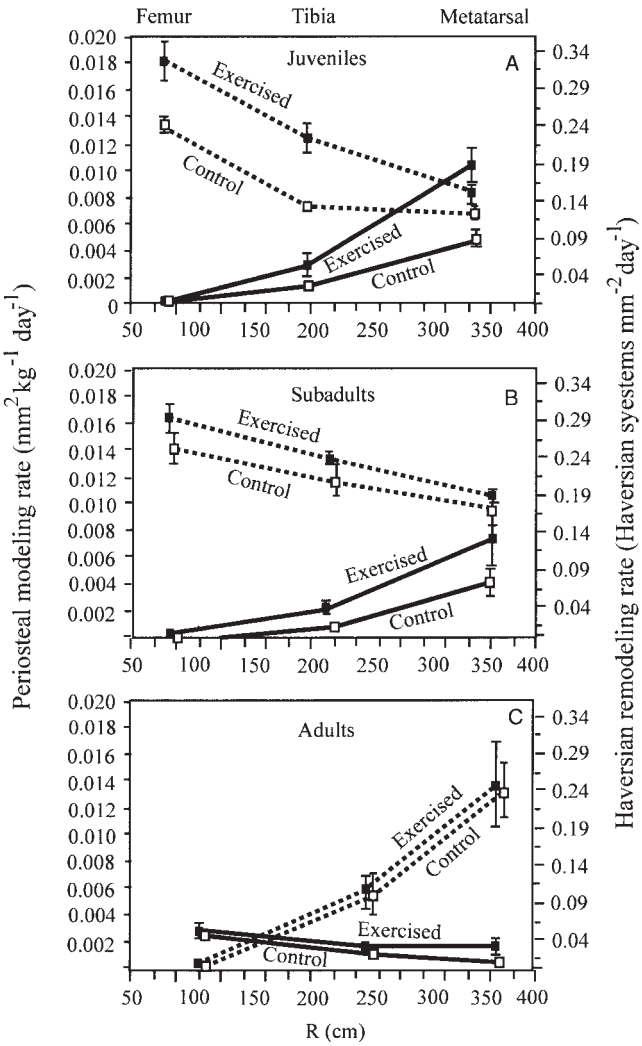

Fig. 9. Experimentally observed trade-offs in sheep hindlimb (modified from Lieberman et al., 2003).

The results of the experiments (Lieberman and Pearson, 2001; Lieberman et al., 2001, 2003) supported the model of Lieberman and Crompton (1998), but also produced some surprises. The juvenile sheep showed great differences between exercising and control animals in modeling and remodeling, while adults showed less modeling and more remodeling overall, with less difference in the responses of exercising and control animals (Fig. 9). The exercising animals in each age group added less subperiosteal bone in the distal segments of their

TABLE 2. Effects of exercise by age in Dorset sheep[1]

Age	Femur			Metatarsal		
	Exercise	Control	Difference[2]	Exercise	Control	Difference[2]
Periosteal bone added (mm^2kg^{-1})						
Juveniles	1.61	1.19	0.42*	0.74	0.61	0.13
Subadults	1.44	1.25	0.19	0.91	0.84	0.07
Adults	0.27	0.24	0.03	0.16	0.07	0.09
Added Haversian density (secondary osteons/mm^2)						
Juveniles	0.05	0.04	0.01	16.31	7.89	8.42*
Subadults	0.05	0.04	0.01	11.08	6.33	4.75
Adults	0.40	0.42	−0.02	22.02	21.42	0.60

[1] Compiled from data for group means from Lieberman et al. (2003).
[2] Difference = exercise − control.
* Significant at $P < 0.05$ via Mann-Whitney U-test.

hindlimb than in the femur. However, the ability of the exercise intervention to produce a difference in the amount of subperiosteal apposition in the midshafts of the femur and metatarsal was 14-fold and 1.6-fold stronger, respectively, in juveniles relative to adults (Table 2). The results of counts of secondary osteons that had been formed or partially completed during the 90-day period also demonstrated a trade-off in all animals. The more distal segments had higher densities of secondary osteons than proximal segments, but the ability of the exercise to increase the rate of Haversian remodeling in the midshafts of the bones was much greater in juveniles than in adults. All of the sheep showed practically no difference between exercise and control groups in the number of secondary osteons/mm^2 added in the femur, but exercise again produced a 14-fold greater respose in increased Haversian density of the metatarsal midshaft in juveniles relative to adults (Table 2). Despite the decreased ability of exercise to stimulate increases in Haversian remodeling in older animals, exercising and control adults had 8-fold and 10.5-fold greater rates, respectively, of Haversian remodeling in their femora than exercise-matched juveniles, and 1.4-fold and 2.7-fold greater rates, respectively, in their metatarsals.

While Lieberman et al. (2003) did find a higher rate of remodeling in distal limbs in somewhat inverse proportion to modeling (as predicted by the mechanostat), the pattern was the reverse of the mechanostat's predictions in terms of mechanical strain thresholds, as Haversian remodeling rates were higher in distal midshafts (namely the metatarsal) subject to higher rather than lower bending and compressive strains than more proximal midshafts (the tibia). Thus the findings of Lieberman et al. (2003) are most in line with the predictions of the optimization theory. One problem with these data, however, is that while Haversian remodeling rates increased in adults, they were not affected by mechanical loading. There are several explanations for this phenomenon. One possibility is that loading levels were not high enough to generate microcracks that were shown to be induced by loading in other studies (e.g., Mori and Burr, 1993; Lees et al., 2002). An alternative explanation is that, like modeling, Haversian remodeling (HR) is also subject to senescence that impairs mechanotransduction so that loading ceases to be able to easily stimulate Haversian remodeling after skeletal maturity. This raises the possibility that HR in adults is more an adaptation to prevent structural damage than a response to strain and/or fatigue damage (Lieberman et al., 2003). These results thus raise questions about how closely humans follow similar age-related declines in responsiveness to exercise. In the case of humans, however, most of the available data concerns changes in bone mineral density (BMD) with execise and age; a smaller subset of experimental data illuminates the effects of exercise on the cross-sectional geometry of long bones.

The evidence for age-related changes in rates of Haversian remodeling is sparse. Relatively few controlled, experimental studies have monitored rates of Haversian remodeling (e.g., Jowsley, 1960; Sedlin and Frost, 1963; Sedin et al., 1963; Frost, 1964; Heřt et al., 1972; Ortner, 1975; Thompson, 1980; Jee et al., 1991; Stout and Paine, 1994; Bouvier and Hylander, 1996; Vashishth et al., 2000; Frank et al., 2002; Lieberman et al., 2003). In humans, some data are available for numbers of secondary osteons vs. age at various skeletal locations (Stout, 1995), and these show substantial variation between sites. On average, it appears that about 5% of the bone mass in long bone cortices is removed and replaced each year in adulthood (Martin et al., 1998). Rates of bone turnover in cancellous bones typically average about 25% per year in humans and in unusual cases may reach up to 200% per year, as measured in the lumbar vertebrae of dogs (Martin et al., 1998). The analysis by Frost (1964) of formation frequencies of secondary osteons in human ribs found a linear decline from birth into early adulthood, approximating zero at age 35. After 35, formation rates increased modestly until age 60, when they equaled rates of formation in the mid-20s, and then declined again.

Additional relevant data come from studies of changes in serum concentrations of biochemical markers of bone formation and resorption in humans and other mammals. Most of these studies were conducted on young individuals in order to understand the effects of specific types of loading on

bone formation rates, and thus are of limited utility for characterizing age-related changes across the life span. In addition, because serum concentrations represent an average of the entire body, these studies also do not reveal disparities between rates of remodeling at different skeletal locations. Nevertheless, studies of biochemical indications of modeling and remodeling provide some interesting information. In one study, Rong et al. (1997) compared the effects of endurance and strength training on serum calcitonin, parathyroid hormone (PTH), osteocalcin, and procollagen type-I C-terminal molecules (ICTP, a marker of collagen catabolism from osteoclastic activity) in eight healthy human males aged 23 ± 3 years. The endurance training consisted of 45-min sessions of pedaling a cycle ergonometer at 55% of maximum oxygen consumption (VO_{2max}), while the strength training comprised 15-min sessions of three sets of leg presses at 85% VO_{2max}. Blood from eight nonexercising controls was drawn and analyzed at the same time as blood from the exercising individuals. Concentrations of the biochemicals in the blood were measured before, 4 hr after, and 24 hr after each bout of exercise. PTH levels increased after strength training but not after endurance training, while osteocalcin levels rose after endurance training but not after strength training. ICTP showed a pronounced decrease after both forms of exercise, indicating a decrease in bone resorption. No change was detected in the concentration of calcitonin. Osteocalcin levels in the strength-training individuals did not differ from controls, but following endurance training, osteocalcin did not show a decline 4 hr later (at around noon) as it did in the controls. Thus both types of exercise appeared to inhibit osteoclastic activity, but the endurance exercise appeared to also increase osteoblastic activity.

Many studies show that weight training is a particularly effective form of exercise for building strong, dense bones. Fujimura et al. (1997) studied the effects of a 4-month regimen of resistance training, which consisted of 45 min of weight training three times per week, in 23–31-year-old males. The study showed that the exercise routine caused a sustained increase in the concentration of the level of serum osteocalcin and bone-specific alkaline phosphatase, but did not produce a change in the level of ICTP relative to controls. Fujimura et al. (1997) concluded that the weight-training program increased bone formation without an initial or sustained increase in bone resorption, a phenomenon reported by a variety of other studies (e.g., Welsh et al., 1997). Woitge et al. (1998) also reported that in 20–29-year-old males, anaerobic exercise (a combination of sprints and weight training) produced elevated levels of serum osteocalcin and bone-specific alkaline phosphatase, but that endurance training (40–60-min runs) produced reductions in the concentrations of these molecules; controls showed no changes. Woitge et al. (1998) concluded that the anaerobic regimen built bone, but that the endurance training produced net bone loss. Unfortunately, Woitge et al. (1998) monitored their subjects for only 8 weeks, thus potentially missing a large component of the osteogenic portion of Haversian remodeling (Martin and Burr, 1989; Martin et al., 1998).

Evidence for interactions between age and loading on modeling

Measures of cross-sectional geometry provide the best way of assessing the summed effects of modeling on a bone and provide a good indication of the bone's ability to resist mechnical loads (Martin et al., 1998; Currey, 2002). A variety of studies, summarized below, document how long bones' cross-sectional geometry models in response to exercise, and a smaller subset of these studies provides insights into how response to activity varies across the life span. A much larger body of data from clinical studies of bone mineral density (BMD) provides additional information about how modeling in response to exercise varies with age. For a variety of reasons (see below), data on BMD are less useful than measures of cross-sectional geometry, and often do not provide information about what surfaces on a bone have grown or been resorbed in response to changed mechanical loading.

In general, studies of both cross-sectional geometry and bone mineral density support the mechanobiology model (Carter and Beaupré, 2001). However, some studies also produced unexpected findings, such as changes between periosteal and endosteal modeling and variations between how different sites in the diaphysis of the humerus respond to loading during different times in the life span. The other major finding from studies of BMD is that bones undergo a profound decline with age in their responsiveness to mechanical loads from exercise, a decline that corresponds to the "biological" component of bony responses of Carter and Beaupré (2001). The decline in this "biological" component may be stronger than Carter and Beaupré (2001) envisioned, and its influence during the adolescent growth spurt may be substantially stronger. Unfortunately, the data from these studies are rarely suited for testing optimization models such as the mechanostat (Frost, 1987, 1990) or the trade-off model (Lieberman and Crompton, 1998) because they either 1) consider only clinically important sites such as the lumbar spine and femoral neck, or 2) do not compare midshafts of multiple long bones within a single limb. Despite the limited ability of these studies to test models for why the skeleton models the way it does, the age-related patterns of change in skeletal modeling in response to exercise are still of interest and are detailed below.

Cross-sectional geometry

If bones function as beams (see above) and model to resist bending, twisting, and compression, then the cross-sectional geometry of an animal's long

bones should be a good indicator of the mechanical forces that the animal had adapted to resist in life, and thus a reasonable reflection of habital activities (e.g., Alexander 1977; Currey and Alexander, 1985; Demes et al., 1991; Ruff, 2002; Polk et al., 2000). Early applications of beam mechanics to model the strength of human long bones were made by Pauwels (1980), Endo and Kimura (1970), Kimura (1974), Lovejoy et al. (1976), and Lovejoy and Trinkaus (1980), among others. Such studies have become more frequent since the development of technology to digitize the cross-sections of long bones and of computer programs like SLICE (Nagurka and Hayes, 1980) and NIH Image that can rapidly calculate second momements of area, facilitating many recent studies of cross-sectional geometry (e.g., Ruff et al., 1999, 2000; Ruff, 2000a; Trinkaus and Ruff, 1999a,b; Ledger et al., 2000; Trinkaus et al., 1999; Churchill and Formicola, 1997; Holliday, 1997; Stock and Pfeiffer, 2001).

Ontogeny of diaphyseal cross-sections

Studies of cross-sectional geometry provide considerable evidence for important age-related effects on modeling responses to mechanical loading. Most importantly, as the mechanobiology model proposes, long bone diaphyses grow thicker and stronger as bone length and body mass increases (van der Meulen et al., 1993, 1996; Ruff et al., 1994; Carter et al., 1996; Carter and Beaupré, 2001). In fact, body mass emerges as the strongest single predictor of femoral cross-sectional geometry, explaining 82% of the variance in femoral second moments of area (van der Meulen et al., 1996). As children progress through the adolescent growth spurt, the peak rate of growth in mass lags behind the peak rate of growth in stature, so that most adolescents intitially become tall and thin and later fill out. The growth of diaphyseal cross-sections largely reflects the later increase in body mass (Ruff et al., 1994; van der Meulen et al., 1996). Uusi-Rasi et al. (1997) reported the accrual of body mass to be the single most important correlate of increasing bone mineral content, bone mineral density, and volumetric bone mineral density in Finnish girls from 8–20 years of age. Although well-nourished European women do not obtain their peak bone mass until 20–26 years of age (Teegarden et al, 1995; Haapasalo et al., 1996a), Theintz et al (1992) reported that the accumulation of bone in the femoral neck of Swiss girls decceleratated rapidly after 16 years.

Recent studies of peripubescent tennis players and younger children who engage in physical activities have modified the descriptive synthesis by Ruff et al. (1994) of age-related changes of bone deposition beneath the periosteum and endosteum (Haapasalo et al., 1996b, 1998, 2000; Bass et al., 2002; Kontulainen et al., 2003). These studies indicate that exercise before the adolescent growth spurt produces fairly subtle modifications in the size of children's long bone cross-sections and that, like adults and children near the end of the adolescent growth spurt, exercising children primarily add bone on the endosteal surface of their cross-sections (Bass et al., 2002). Children's bones may "add" bone endosteally by slowing their baseline rate of endosteal resorption, whereas late adolescents and young adults appear to actively deposit new endosteal bone. Bass et al. (2002) further noted that different parts of the humerus appear to respond to loading in different ways from childhood through late adolescence (Fig. 10). In particular, in late adolescence, the distal (80% of the length) shaft of the humerus experiences more endosteal modeling in response to loading than the midshaft.

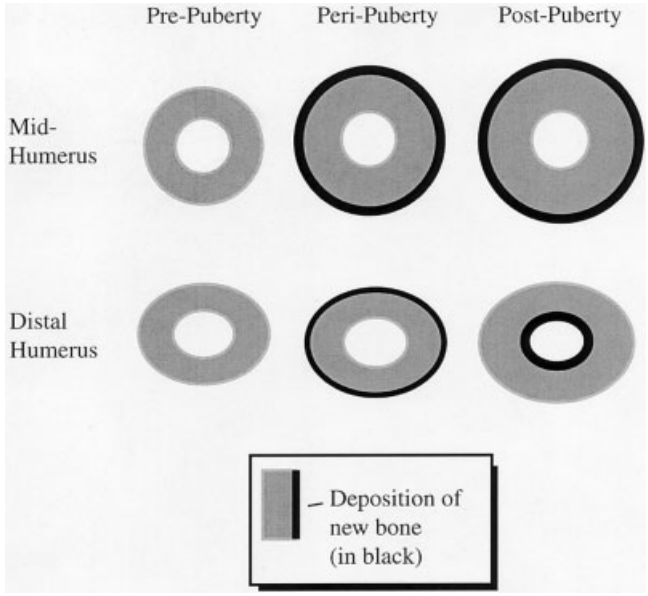

Fig. 10. Model by Bass et al. (2002) of changes with age in bone modeling at different sites in humerus.

The age-related pattern of changes in responsiveness in the periosteal and endosteal surfaces of long bones may also apply to the bones of the lower limb. In a study of the ability of an exercise intervention to build bone in prepubertal boys aged 8.4–11.8 years, Bradney et al. (1998) reported that exercising boys gained significantly more bone mineral density (BMD, g/cm^2) than nonexercising boys in all measured skeletal sites except the head and arms, and that the increases in BMD primarily resulted from an increase in cortical thickness via less endosteal resorption rather than an increased rate of deposition of subperiosteal bone.

Several studies also showed impressive effects of vigorous exercise on cross-sectional properties of the humerus during the adolescent growth spurt. The most famous of these is Jones et al. (1977), which was reanalyzed by Trinkaus et al. (1994) and Ruff et al. (1994). Jones et al. (1977) found that male tennis players had on average a 35% larger cortical thickness of their dominant arm, while female players' dominant arm had 29% thicker cortical bone that

TABLE 3. Humeral asymmetry in tennis players[1]

Scan level/variable	Asymmetry in players	Asymmetry in controls
Humeral shaft		
I_{min}	46.4 ± 36.9%	5.2 ± 31.2%
I_{max}	39.0 ± 17.4%	16.1 ± 12.4%
Cortical wall thickness	20.2 ± 10.5%	0.4 ± 3.1%
Distal humerus		
I_{min}	37.4 ± 21.8%	16.3 ± 13.9%
I_{max}	67.0 ± 37.7%	5.7 ± 18.0%
Cortical wall thickness	24.6 ± 8.8%	4.6 ± 4.3%
Radial shaft		
I_{min}	60.8 ± 46.6%	5.3 ± 14.5%
I_{max}	34.6 ± 20.9%	6.7 ± 8.6%
Cortical wall thickness	4.5 ± 6.5%	−0.1 ± 5.6%

[1] Data from Haapasalo et al. (2000).

their nondominant arm. Bertram and Swartz (1991) proposed that much of the difference that Jones et al. (1977) had found between the tennis players' arms was due to injury and pathological inflammation of the dominant arms rather than loading, citing the fact that 45% of the players in the study had experienced chronic pain in their dominant arm. More recently, however, Haapasalo et al. (2000) used peripheral quantitative computed tomography (pQCT) to study bilateral asymmetry in the humerus and radius of 12 male former Finnish national-level tennis players (aged 29.8 ± 4.8 years) with 12 age-, weight-, and height-matched controls. Subjects were not recruited for the study because they had chronic elbow pain, thereby controlling for the criticism by Bertram and Swartz (1991) of Jones et al. (1977). The tennis players in Haapasalo et al. (2000) had started training at 10 ± 3 years, played for an average of 19.6 ± 5.3 years, and still played recreationally about three times per week. Haapasalo et al. (2000) reported that the elite players had substantially more asymmetry in the second moments of area (I_{max} and I_{min}) of the mid- and distal humerus than nontennis-playing controls, but with a substantial amount of variability, which suggests the importance of other, uncontrolled factors (Table 3). A similar study by Kontulainen et al. (2003) that used pQCT to assess cross-sectional properties in the humeri of female tennis and squash players also found a substantial increase in asymmetry in the tennis players over controls, with markedly greater asymmetry in girls who had started training at or before menarche. Likewise, Bass et al (2002) used a combination of photon absorptiometry and MRI to gauge the effects of tennis training on the humeral cross-sections of 47 female competitive tennis players, aged 8–17 years. The girls were divided into prepubertal (n = 17), pubertal (n = 11), and postpubertal (n = 19) groups. Bass et al. (2002) found that asymmetry in the dominant vs. nondominant arm increased with age, but that the largest amount of increase in asymmetry in the polar second moment of area (J) of the humeral midshaft developed between the prepubertal and pubertal groups, with no subsequent increase. Asymmetry in J in the distal humerus continued to increase into the postpubertal period (Fig. 10).

Important supplements to the human studies summarized above are numerous experimental studies in animals that examined the effects of mechanical loading on modeling (for recent and comprehensive reviews, see Goodship and Cunningham, 2001; Currey, 2002). Most animal studies examined either juvenile or adult animals, and few compared animals at different ontogenetic stages. This issue, however, was investigated explicitly and in detail by Lieberman et al. (2003), who compared sheep of different ontogenetic ages. This study found a strong effect of exercise on periosteal modeling in young individuals, a decreased effect in subadult animals, and no measurable effect on adults (Fig. 9). In addition, there was no evidence for significant endosteal apposition at any age; however, because Lieberman et al. (2003) measured cross-sectional geometry using fluorescent dyes, they were unable to assess rates of endosteal resorption. Interestingly, these results contrast with those of Woo et al. (1981), who found that exercise inhibited endosteal resorption but did not stimulate periosteal growth in the femora of growing miniature swine exercised for 6 km/day for 1 year. In another study using swine, Lieberman (1996) and Lieberman and Crompton (1998), however, found that exercise did significantly increase cortical bone growth in limb midshafts of miniature swine exercised twice daily for 30 min each for 90 days compared to controls. Further research is necessary to understand these differences.

One additionally interesting result from animal experiments is that the addition of mass in the diaphysis may not always be related in a simple manner to the mechanical function of the midshaft (as Wolff's "law" might predict). Several studies, including Lieberman et al (2003, 2004a), Demes et al. (1998, 2001), Gross et al. (1992), and Szivek et al. (1992), applied strain gauges to three locations around the midshaft of long bones. These experiments for the most part confirmed that long bones typically are bent in the sagittal plane during locomotion, but highlighted several complications. First, strains during activities such as locomotion are quite dynamic, with substantial shifts in the orientation and position of the neutral axis. In trotting sheep, for example, the orientation of the neutral axis (NA) rotates about 65° in the tibia and 40° in the metatarsal during stance (Fig. 11). Similar rotations were shown to occur in macaques (Demes et al., 1998, 2001), horses (Gross et al., 1992), and greyhounds (Szivek et al., 1992). In addition, the orientation of the NA is often poorly correlated with the orientation of I_{max}, the axis around which the bone is strongest in resistance to bending. In sheep, for example, the tibia is strongest at resisting mediolateral bending, even though it is mostly bent in the parasagittal plane (Fig. 11) (Lieberman et al., 2004a). Finally, these studies confirm the prediction

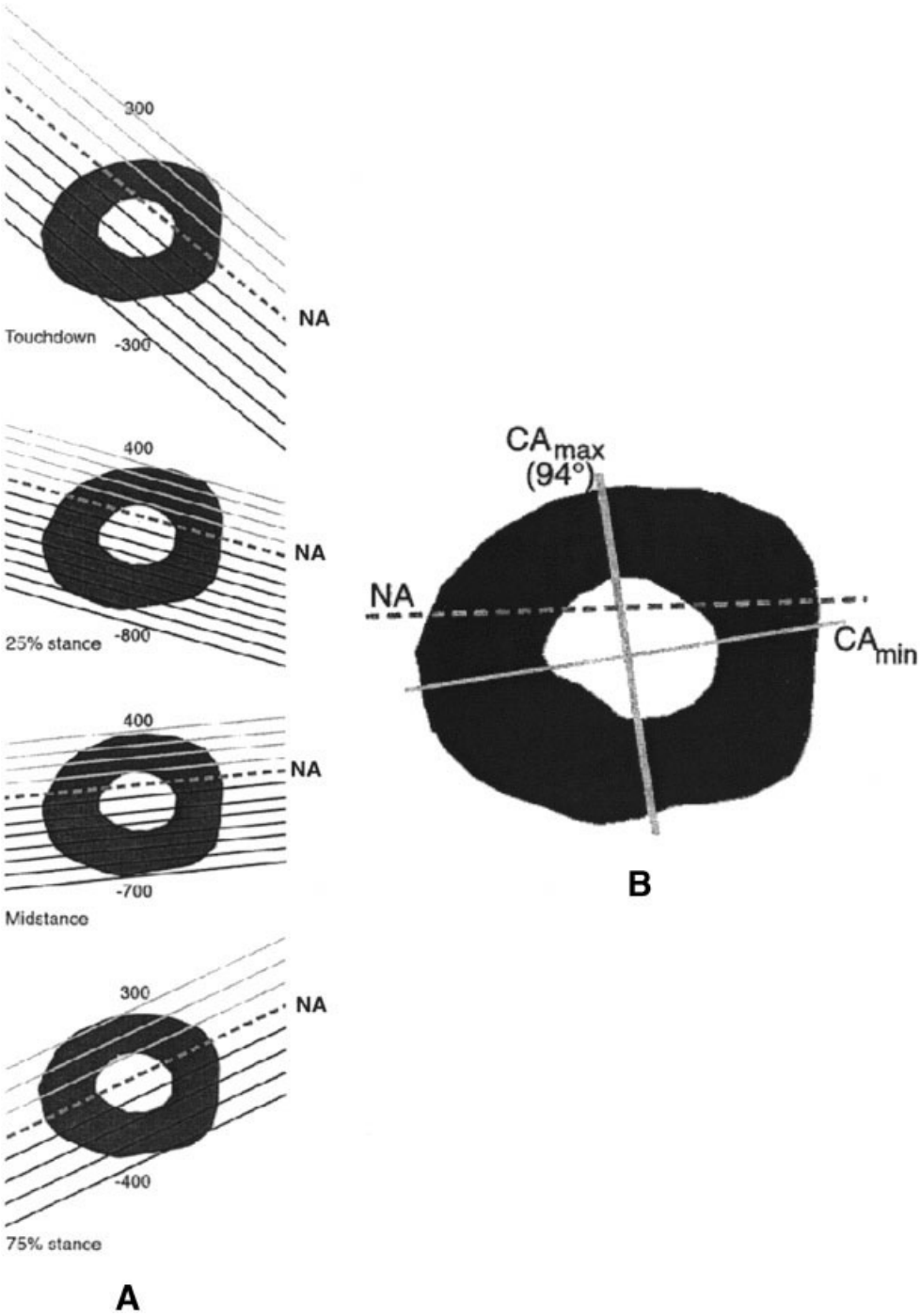

Fig. 11. A: Patterns of strain in sheep tibia throughout gait cycle. Negative strains indicate compression; positive strains denote tension. **B:** Empirically determined axis of bending around neutral axis (NA) of strain in sheep tibia vs. a priori calculated axis (CA) of bending. CA runs through centroid of area and defines axis around which bone section has greatest second moment of area. Note that actual axis of bending does not pass through centroid of area, and that during locomotion, tibia is bent almost perpendicularly to direction in which it is mechanically best suited to resist bending.

(Lovejoy et al., 2003) that the neutral axis does not typically run through the cross-sectional centroid of most bones. Long bones are mostly bent, but they also experience substantial amounts of axial compression, which superimposed on bending will always shift the NA toward the cortex subject to tension, depending on the relative percentage of compression. As a result, calculations of second moments of area which assume that the NA runs through the area centroid may have considerable error, because I is calculated using the square of the distance of any given area of bone relative to the assumed or known neutral axis. Lieberman et al. (2004a) found that for trotting speeds, estimates of various cross-sectional properties were in error by 23–38%. It remains to be tested if these errors decline with increasing speed or in galloping vs. trotting; if so, then the implication would be that bone cross-sectional properties mostly reflect shape adaptations to only the most vigorous forms of loading rather than typical habitual activities. Although these studies on the effects of exercise on cross-

sectional geometry are most relevant to the large amount of work that anthropologists have done on the cross-sectional geometry of prehistoric populations, there are many additional studies of bone density that also provide useful information about the way in which skeletal responses to exercise vary across the life span.

Bone mineral density

A great wealth of data has been accumulated from clinical studies of bone mineral density (BMD) that is useful for investigating the interaction between aging and exrcise (loading) on skeletal modeling and remodeling. Unfortunately, in many cases, the density data are less useful for understanding precisely where additional bone has been added or subtracted in response to exercise. Most of the clinical research employed photon absorptiometry to measure bone quality and reported their findings in terms of bone mineral density (BMD, measured as g/cm^2) or bone mineral content (BMC, measured as grams of bone). Unfortunately, the heavy reliance upon photon absorptiometry in the clinical literature is problematic in the present context, because the "density" values derived from photon absorptiometry depend on having an accurate model of the periosteal and endosteal contours of the bone in question; otherwise, measures of BMD and BMC may not accurately reflect the actual density (g/cm^3) or the bending strength of the bone (Lam et al., 1998, 2003; Sievänen et al., 1998; Haapasalo et al., 2000; Kardinaal et al., 2000). Direct comparisons of measures of BMD and BMC between subjects work well only if the periosteal and endosteal contours of the individuals' bones remain constant or nearly so. Changing the shape of bones in these studies has the effect of either increasing or decreasing the apparent BMD and BMC, thus distorting comparisons (for additional details, see Lam et al., 2003). With these caveats in mind, however, the clinical literature on BMD reveals a variety of interesting things about the influence of exercise on bone.

Experimental studies of the effects of exercise show that high-impact sports produce the highest BMD, particularly sports that involve sprinting or other short-duration, high-intensity forms of exertion (i.e, gymnastics, weight-lifting, jumping, or soccer; Alfredson et al., 1996, 1997, 1998a,b; Fehling et al., 1995; Bennell et al., 1997; Wittich et al., 1998; Mayoux-Benhamou et al., 1999; Nordström et al., 1997; Söderman et al., 2000; Pettersson et al., 1999a, 2000a,b; Heinonen et al., 2002). Lower-impact endurance sports produce lower BMD. Swimming, which involves strenuous but prolonged exertion in a very low-impact environment (water), does not produce markedly elevated BMD (Nilsson and Westlin, 1971), although it does increase BMD (Orwoll et al., 1987). Studies of adults and children found that BMD is correlated with muscle strength (Nilsson and Westlin, 1971; Dalén and Olsson, 1974; Pocock et al., 1987; Karlsson et al., 1993; Menkes et al., 1993; Düppe et al., 1997; Nordström et al., 1996, 1998; Tsuzuku et al., 1998; Söderman et al., 2000; Pettersson et al., 2000b; Ruff, 2003b; Capozza et al., 2004; Daly et al., 2004). Experimental investigations in myostatin knockout mice, which develop abnormally large muscles as a result of the loss of function of the myostatin gene, produce even more dramatic associations between muscle and bone mass (Hamrick, 2003; Hamrick et al., 2002, 2003). Furthermore, numerous studies documented that exercise has the greatest ability to produce increases in bone mass and density in skeletal sites most directly affected by impact forces or specific muscular contractions (Slemenda and Johnston, 1993; Nordström and Lorentzon, 1996; Söderman et al., 2000; Heinonen et al., 2002; Iuliano-Burns et al., 2003). One exception to this pattern lies in the fact that amenorrheic runners have low bone density throughout their skeleton, including the bones of their lower limbs (Pettersson et al., 1999b), although these runners (and some ballerinas, bulimics, and anorexics) also tend to experience confounding physiological changes, including low levels of estrogen and chronically low levels of energy.

Exercise and BMD in childhood and adolescence

Since the mid-1990s, it has become increasingly apparent that there are substantial age-related differences in the body's ability to increase BMD in response to exercise. In girls and boys, the adolescent growth spurt, especially its early stages, appears to be a major window of opportunity to build bone. Finnish scientists produced one of the best series of studies on this phenomenon (Kannus et al., 1994, 1995; Haapasalo et al., 1996a,b, 1998, 2000; Haapasalo, 1998; Heinonen et al., 1996, 1999; Kontulainen et al., 1999, 2001, 2003). These studies documented the development of bone density, mass, and asymmetry in the humeri of young women who played tennis and squash and in control subjects of similar age. The Finnish studies were able to document the effects of different starting ages on bone accrual as well as the rate at which bones changed after the cessation of training.

The Finnish studies found that the age at which girls started playing tennis had a huge impact on humeral asymmetry. Girls who started training before menarche obtained twice the asymmetry in BMC than girls who started playing after menarche and three times the asymmetry of controls (Kannus et al., 1995; Haapasalo, 1998) (Fig. 12). The best time to start playing tennis in order to build bone appeared to be just before or around menarche, in Tanner stages III–IV (Kannus et al., 1995; Haapasalo, 1998). Girls who started playing tennis between 3–5 years prior to menarche did obtain a higher peak bone mass than girls who started playing between 0–3 years before menarche, but the difference in extra bone mass was not large enough to reach statistical significance. Girls studied during

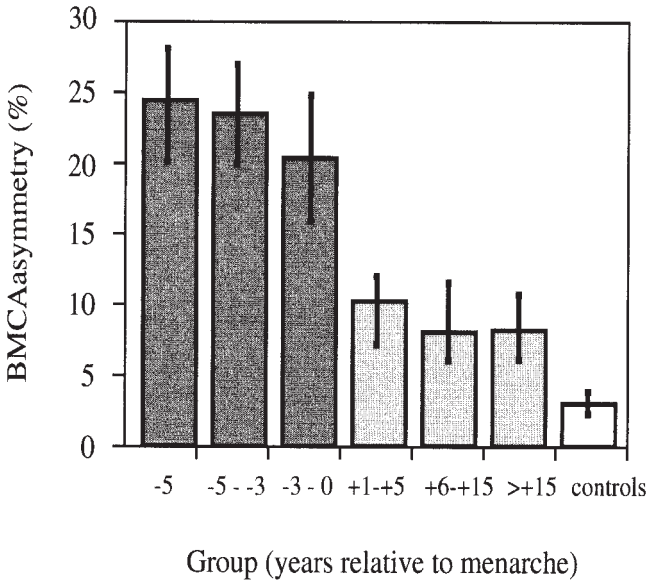

Fig. 12. Effect of starting age on acquired asymmetry of humerus in female tennis players. Bars indicate 95% confidence intervals (after Haapasalo, 1998).

Tanner stage I (about 9 years old) had not developed much asymmetry as a result of training (Haapasalo, 1998; Haapasalo et al., 1998). From photon densitometry images of humeri, Haapasalo et al. (1996b) calculated BMC, BMD, bone area, and estimated second moment of area and section modulus (Z) of the humeri of young tennis-playing men (n = 17) and women (n = 30) relative to controls (n = 16 and 25, respectively). The young men and women had much larger values than controls for asymmety in BMD, cortical width, and second moments of area of the humerus (Table 4). In the case of BMD, the results indicated substantial differences in asymmetry between the tennis players and controls, with increasingly large and distinctive amounts of asymmetry in BMD in the more distal part of the shaft. A subsequent study (Haapasalo et al., 2000) of tennis players' humeri using peripheral quantitative computed tomography (Gasser, 1995; Sievänen et al., 1998) found that the apparent difference in BMD was created by changes in bone dimensions rather than actual (volumetric) density of the bone, a finding that is important to bear in mind with regard to all the discussion that follows of differences in BMD due to activity. Similar findings for tennis players were reported by Bass et al. (2002). Follow-up studies (Kontulainen et al., 1999, 2001) addressed how much of the bone mass and size built during the adolescent growth spurt was preseved during the next 4–5 years.

Other researchers found similar effects of age and exercise in ballerinas (K. Khan et al., 1998) and gymnasts (Bass et al., 1998), and with a variety of other exercise interventions (K. Khan et al., 2000; French et al., 2000; Bass et al., 2002; Sundberg et al., 2002; Gustavsson et al., 2003; Kontulainen et al., 2003). Although some studies produced exceptions, the majority showed that the adolescent growth spurt provides children with a window of opportunity to build thick, strong bones. Uncertainty remains over how effectively activity earlier in childhood or later (after the adolescent growth spurt or at its tail end) can build bone. Studies of the effects of activity in prepubescent children offer condradictory evidence. Some found that training in high-intensity sports like gymnastics or jumping exercise produced modest increases (about +3%) over controls in BMD in prepubescent children (Daly et al., 2004; Specker and Brinkley, 2003; Johannsen et al., 2003; Scerpella et al., 2003; MacKelvie et al., 2001, 2004; Heinonen et al., 2000; McKay et al., 2000; Fuchs et al., 2001; Morris et al., 1997; Cassell et al., 1996). Other studies found exercise regimes to produce few significant diffences in prepubescent children (Van Langendonck et al., 2003; Witzke and Snow, 2000).

Exercise in late adolescence following puberty produces additional growth in cortical bone, but to a lesser extent than exercise during the adolescent growth spurt. In one study of 12–16-year-old students in Sweden, only males benefited from an exercise intervention (Sundberg et al., 2001). In another study, 26 weeks of weight training produced no significant increase in lumbar or total-body BMD in a sample of 14–18-year-old girls (Blimkie et al., 1996). Valdimarsson et al. (1999) found that lean body mass was a better predictor of BMD than weight, height, or fat mass in young women aged 16–20 from Reykjavik, but the study also found that BMD increased logarithmically with hours spent exercising per week (as assessed via a questionnaire developed by Slemenda et al., 1991), and that 31.2% of the variance in total-skeleton BMD in the women could be explained by lean body mass and amount of exercise.

Growth in muscle size (usually measured in terms of the cross-sectional area of muscle at a given spot in the limb) provides an important correlate of bone mass. Muscle growth follows the growth curve for growth in mass, and thus lags behind the growth-velocity curves for stature (Ruff, 2003b; Daly et al., 2004; Rausch et al., 2004). Bone mineral content (BMC) of the limb bone reaches peak growth velocities between 0.71–0.36 years after the maximum growth velocities for muscle areas in the limbs in both sexes, reinforcing the idea that bones adapt to the additional loads that larger muscles are able to create (Rausch et al., 2004). However, in a similar longitudinal study as that of Rausch et al (2004), Ruff (2003b) found a much stronger correlation between arm muscle area and humeral section modulus in adolescent males than females; femoral strength was closely correlated with growth in body mass in both sexes.

Of these three studies, however, only Daly et al (2004) were able to check for additional effects attributable to exercise during growth. Daly et al. (2004) studied the playing vs. nonplaying arm in 47

TABLE 4. Humeral asymmetry at various sites in male and female tennis players and controls[1]

	Young female players	Young female controls	Young male players	Young male controls
BMD (g/cm^2)				
Proximal	15.4 ± 5.3%	1.7 ± 3.3%	17.3 ± 7.3%	1.9 ± 3.7%
Middle	16.1 ± 7.7%	0.5 ± 2.7%	21.8 ± 6.2%	1.8 ± 2.6%
Distal	19.6 ± 7.3%	3.9 ± 3.6%	22.5 ± 8.4%	2.6 ± 3.1%
CWT				
Proximal	17.9 ± 6.8%	1.7 ± 4.5%	20.0 ± 8.9%	2.1 ± 4.3%
Middle	20.4 ± 10.5%	0.4 ± 3.5%	29.7 ± 9.1%	1.8 ± 2.9%
Distal	30.9 ± 12.1%	5.9 ± 5.8%	45.2 ± 19.2%	3.9 ± 7.0%
SMA				
Proximal	23.8 ± 13.9%	6.5 ± 11.6%	26.4 ± 13.6%	5.7 ± 14.1%
Middle	15.4 ± 9.6%	4.2 ± 7.3%	16.6 ± 14.8%	6.5 ± 11.6%
Distal	14.1 ± 11.3%	1.0 ± 7.9%	16.0 ± 7.1%	8.2 ± 13.6%

[1] Data from Haapasalo et al. (1996b).
CWT, cortical wall thickness.

female competitive tennis players aged 8–17 years, divided into pre-, peri-, and postpubertal groups. In the nonplaying arm, both groups showed moderate to high correlations ($0.56 \leq r \leq 0.81$) between muscle area and various measures of humeral cross-sectional size and strength. However, relative to muscle area, postpubertal players had thicker humeral cortices and relatively contracted medullary cavities compared to younger players. Daly et al. (2004) did not find a major increase in the asymmetry of the humeri around the time of puberty, unlike a series of other recent studies, although they did find the same postpubescent pattern of endosteal deposition that Bass et al. (2002) described. The reason for the disparity between the studies in the development of humeral asymmetry remains unknown. On the whole, most studies reported that the time around the growth spurt constitutes a sensitive period for building bone mass in both boys and girls, but more research is needed.

Exercise and BMD in adulthood

Exercise in adulthood after the skeleton has matured appears to make less of an impact on skeletal form or cortical thickness. Nevertheless, some studies showed that bone mass added as a response to exercise can have lasting benefits for the skeleton (e.g., Teegarden et al., 1996; Mickelsfield et al., 2003). However, other studies showed that much of the bone mass that adults add as the result of beginning an exercise regimen is rapidly lost after the exercise program ends. Bone added during the adolescent growth spurt may convey more lasting skeletal strength, although some studies reported contradictradictory findings in this regard. Welten et al. (1994) reported on a 15-year longitudinal study of 84 men and 98 women from Amsterdam whose daily calcium intake, body mass, and weight-bearing activity had been monitored from 13–28 years of age. In multiple regression analyses, Welten et al. (1994) found that only weight-bearing activity and body mass proved to be significant predictors of lumbar BMD at 27. Similarly, Groothausen et al. (1997) found that 25% of the variance in lumbar spine BMD at 27 is related to peak strains (from high-impact physical activities) in the previous 15 years.

In addition, surveys of the effect of lifetime physical activity on BMD in older adults produced mixed results, some of which suggest that activity during (or at least in the period around) the adolescent growth spurt exerts a measurable influence on BMD decades later, while others do not. Kriska et al. (1988) found that historical activity had low but significant correlations with bone density and bone area ($r = 0.16$, $P < 0.05$ in both cases) in postmenopausal women. In addition to other periods in the life span, Kriska et al. (1988) assessed physical activity in the period from 14–21 (i.e., just after to several years after the probable time of menarche for most of the women in the study), finding that exercise in that period had a low ($r = 0.14$, $P < 0.05$) but significant correlation with current bone area in the radius. Greendale et al. (1995) found that historical activity (including activity in the "teenage years," i.e., around and after menarche) did not correspond to significant differences in the BMD of the radius, wrist, or spine, but did account for significant differences in current BMD in the hip and proximal femur in seniors (average age, 73) from California. Ulrich et al. (1999) studied the effect of lifetime weight-bearing physical activity on BMD in 25 premenopausal women aged 28–50 years, and found a higher correlation ($r = 0.54$, $P < 0.05$) between previous activity and total-body BMD than reported by many other studies, many of which had also been conducted on older individuals. Ulrich et al. (1999) reported that activity during early childhood (6–12 years of age) and the teenage years (13–19 years of age) produced marginally higer correlations with current total-body BMD than activity over the last 2 years ($r = 0.38$, $r = 0.40$, and $r = 0.31$, respectively). Brahm et al. (1998) investigated the relationship between lifetime physical activity and bone mass in 61 men and 61 women, aged 22–85. After adjusting for smoking, milk consumption, age relative to menopause, and use of hormone replacement therapy, Brahm et al. (1998) found no significant difference in BMD among the most-active to

least-active quartiles of 22–85-year-old women and men. However, lifetime physical activity had a greater impact on bone area (width), especially in males. Unfortunately, Brahm et al. (1998) did not investigate the effect of activity during the adolescence growth spurt per se. Karlsson et al. (1995) studied bone mass in 48 male ex-weight-lifters, aged 50–79 years, who had lifted for an average of 13 years and retired by age 30. The younger (50–64 years) ex-weight-lifters retained higher BMD than controls, but older ex-weight-lifters did not. The results of Karlsson et al. (1995) are also more applicable to the lasting effects of exercise in young adulthood rather than around the time of the adolescent growth spurt.

Karlsson et al. (2001) found little if any reduction in risk of osteoporotic fractures in people who had been active early in life. Part of the apparent lack of benefit of earlier exercise may be due to what Karlsson (2001) termed the double-edged sword of exercise: it builds or maintains bone, but once the exercise stops, the skeleton undergoes a period of accelerated bone loss (Karlsson et al., 1995; Novotny et al., 2001; Ryan and Elahi, 1998). The rapid period of bone loss can be observed even in the off-season in young athletes (Karlsson et al., 2003; Snow et al., 2001) or people on extended bed rest (Zerwekh et al., 1998). By the fourth decade of life, most adults become less active than they were as adolescents or children. Although there is a tendency for people who were active in sports or other physical activities to remain active later in life, the trend is actually quite weak. Rank-order or Pearson's correlation coefficients between quantiles of age-specific activity in early adulthood vs. middle age or old adulthood are low to moderate ($0.1 > r > 0.3$) (Malina, 2001a,b). Thus at least in contemporary society, most adults experience a marked decrease in the physical activities that they once performed that might have acted to slow the rate of bone loss.

Exercise and BMD in old age

All of the previously discussed declines in osteoblastic function and sensitivity to hormonal and other chemical signals combine to make the skeletons of old adults much less able to build new bone in response to exercise. However, periosteal apposition continues slowly on the shafts of long bones, so that the external dimensions of the diaphyses of a 70-year-old's bones may be subtly thicker than they were in young adulthood, but the cortices will be substantially thinner (Lazenby, 1990). Studies of the effects of exercise in older adults, including postmenopausal women, indicate that exercise can act to slow the rate of bone loss, primarily by downregulating osteoclastic activity, but it is much less effective than exercise earlier in life in triggering osteoblastic activity (Heinonen et al., 1998; Wolff et al., 1999; Brooke-Wavell et al., 2001; Blanchet et al., 2003). Hormone replacement therapy (HRT) helps to reduce the rapid loss of bone following menopause in women, but the combination of hormone replacement therapy and exercise is even more effective (Kohrt et al., 1995; Uusi-Rasi et al., 1999). Some recent controvery surrounds a 15-year longitudinal study by Ahlborg et al. (2003), which reported that postmenopausal women showed an acceleration in periosteal deposition in addition to the expected acceleration in endosteal resorption. It is possible that this pattern is sex-specific and may involve estrogen receptor-β, which may influence bone modeling differently in males and females (Windahl et al., 1999). Some low-impact exercises such as nonstrenuous calisthenics or walking produced no benefits to the maintenance of bone mass in old adults, and only higher-impact or more strenuous activities appear to be effective in slowing the rate of bone loss in older adults (Kerr et al., 1996; Heinonen et al., 1998; Uusi-Rasi et al., 1999; Brooke-Wavell et al., 2001; Parkkari et al., 2000).

CONCLUSIONS

An understanding of how bones respond to mechanical loading during life is an essential prerequisite for interpreting what variations in the morphology of the bones of prehistoric people, hominins, and other organisms reveal about their activities in life. For over a century, the concept known as Wolff's "law" has guided functional morphologists in their attempts to infer function from bones' forms. Although this proposition was originally formulated to explain the organization of trabecular bone during growth and development, anatomists have extended it to cortical bone, which forms the subject of this review. It has become increasingly clear that recent conceptions of Wolff's "law" have conflated a variety of different phenomena that lead to deposition or resorption of bone, some of which can be triggered by signals other than (or in addition to) mechanical strains (Bertram and Swartz, 1991). Furtherore, it has also become apparent that ontogenetic age plays an important role in modulating how strongly cortical bone models and remodels in response to mechanical loading (Bertram and Swartz, 1991; Ruff et al., 1994; Lieberman et al., 2003; Holt et al., 2004). Young mammals, including humans, generally exhibit strong modeling and remodeling responses to loading, while old adults exhibit very little or no response to changes in loading. As a result of this complexity, it is probably best to restrict the term "Wolff's law" to just its original meaning. Combining all of the complex and disparate influences on bone modeling and remodeling into a single, vague, and overarching "law" serves only to obscure the complexity of the system and creates a false impression that bone tissue opperates by means of a single, simple mechanism.

Cellular and hormonal mechanisms provide the key to understanding why bones respond to loading in different ways across the life span. Although much remains to be learned about how numerous hormones, receptors, and other signals interact to

modulate the behavior of bone cells, an impressive amount of progress has been made over the last decade (Harada and Rodan, 2003; Boyle et al., 2003; Lee et al., 2003; Turner et al., 2002; Cowin and Moss, 2001; Ryder and Duncan, 2000; Hsieh et al., 1999). Cell senescence accounts for part of the changes in the way that the skeleton responds to mechanical stimuli. Populations of osteoprogenitor cells undergo a marked senescence in adulthood, with a large decline in cell numbers and decreased responsiveness of the remaining cells to mechanical stimuli, estrogen, IGF-I, and TGF-β (Nishida et al., 1999; Muschler et al., 2001; Turner et al., 1995c; Tanaka and Liang, 1996; Ankrom et al., 1998; Bätge et al., 2000). Although the mechanisms responsible for bone modeling and remodeling are not yet completely understood, the pattern of responses across the life span is fairly clear.

Studies of living people show that activities during childhood and particularily during the adolescent growth spurt can play an important role in establishing adult morphology (Haapasalo et al., 1998; K. Khan et al., 2000; Bass et al., 2002; MacKelvie et al., 2002), which subsequently becomes increasingly difficult for the body to change (K. Khan et al., 1998; Kontulainen et al., 1999, 2001), especially as its oseoprogenitor cells senesce (Nishida et al., 1999; Bätge et al., 2000; Muschler et al., 2001; Turner et al., 1995c). The existence of this "window of opportunity" for the body to grow strong, thick (robust) bones has important implications for attempts to interpret skeletal morphology. Much of what we see in an adult's cortical bone morphology is a result of the history of skeletal loading during adolescence. If this assessment is accurate, then osteologists might profitably direct much more attention to the study of the biomechanics of juvenile skeletons and the ontogeny of thick long bone diaphyses, upper limb asymmetry, pronounced muscle marks, and exaggerated shapes of of the cross-sections of long bone diaphyses (e.g., platycnemic tibia and well-developed femoral pilasters). Unfortunately, the study of juvenile remains (especially from a biomechanical perspective) has long received less attention than studies of adults, although there was a recent renaissance of interest in the study of juvenile remains (e.g., Ruff, 2003a; Scheuer and Black, 2000; Clegg and Aiello, 1999; Zilhão and Trinkaus, 2002; Akazawa and Muhesen, 2002; Mednikova and Trinkaus, 2001; Hoppa, 2000; Thompson and Nelson, 2000; Tillier, 1999; Molleson et al., 1998; Trinkaus and Ruff, 1996; Lampl and Johnston, 1996).

Likewise, ethnographers have often not devoted as much time to describing children's activities as they have to those of adults, but the role of physical development as skill-acquisition is also receiving renewed interest (e.g., Walker et al., 2002). Gurven and Kaplan (in press) showed that time allocations devoted to labor activities among the Machiguenga and Piro of Peru rise steeply through childhood. In males, time spent hunting exhibits a particularly rapid rise, peaking at about 26 years of age and subsequently declining. Women show a similarly rapid increase in the time devoted to child care from their own childhood until about 23 years of age, followed by a gradual decrease. Other activities occupy less of adolescents' time or peak later in life. Maturation and the adolescent growth spurt occur somewhat later among the Machiguenga than among well-nourished North Americans with access to modern medical care (Kaplan, personal communication), and so adolescents in the community begin to intensify their participation in behaviors typical of adults in approximate synchrony with their physical maturation. If these patterns accurately characterize other preindustrial societies, then it is likely that adolescents in most of those societies begin to engage intensively in the strenuous activities typical of adults during their adolescent growth spurts, and thus the adult morphology and cross-sectional geometry of their long bones probably would reflect those activities.

Another issue that this review touches upon also stems from a lack of basic, experimentally derived data on how human long bones are actually deformed by mechanical loads. Many researchers have assumed that the shapes of long bone shafts, whether quantified by external measurements or cross-sectional geometry, reflect the habitual types of loads imposed upon the bones. The next step has often been to use the shapes of diaphyseal crossstions of prehistoric people to infer what activities led to the development of the shapes that anthropologists observe. While many of these explanations seem reasonable, we should be aware that a strong empirical basis for such interpretations has been mostly lacking (Jurmain, 1999). The potential magnitude of this problem in interpretation is highlighted by a variety of experimental strain-gauge studies of actual loading patterns, in which limb bones are often bent around axes in vivo that do not correspond to the orientation of maximal stiffness in resistance to bending (Gross et al., 1992; Szivek et al., 1992; Demes et al., 1998, 2001; Lieberman et al., 2004a). These results underscore the need for more such studies in animal models (possibly even humans) and for more caution in extrapolating specific differences in physical activities from variations in cross-sectional geometry.

Biological anthropologists have the opportunity to continue to make substantial contributions to the body of knowledge about how lifestyles affect bones. Given the mounting evidence that activity early in life (especially around the time of the adolescent growth spurt) contributes heavily to the development of adult morphology and diaphyseal cross-sectional dimensions, there is a crucial need for more data on responses to mechanical stresses in children and teenagers, both in living children and in skeletal samples of ancient children. Likewise, more studies are needed of skeletal series to document the development during childhood of bone shape (including

asymmetries), bone thickness, and musculoskeletal stress markers (MSMs) (Hawkey and Merbs, 1995), and more data are needed in general on how and to what extent living people's bones respond to mechanical loading throughout their lives. The last decade has witnessed a rapid increase in our knowledge of how molecular and mechanical stimuli regulate the actions of cells which create the gross morphology that anthropologists have long studied. Investigations of how, why, and when during development physical activities exert influence on the morphology of bones will continue to benefit from advances in bone biology and microbiology. There is cause for optimism that the next decade will bring many improvements in how we infer prehistoric activity patterns and life history from variations in cortical bone morphology and histology.

ACKNOWLEDGMENTS

We thank R. Bernstein, B. Richmond, A. Biewener, A.W. Crompton, B. Demes, M. Devlin, G. Krovitz, K. Mowbray, J. Polk, C. Ruff, and D. Strait for collaborations, discussions, and technical assistance. P.-Y. Ju, J. Smart, H.-M. Lin, J. Fildes, and S. Kohn helped with the references. J. Baker, M. Devlin, and R. Bernstein provided useful comments on early drafts of the manuscript. S. Stinson, M. Hamrick, D. Weaver, and three anonymous reviewers provided many useful criticisms and suggestions. Funding for the experiments reported was provided in part by NSF IBN 96-03833 and a grant from the American Federation for Aging Research to D.E.L., and by George Washington University, Harvard University, and the University of New Mexico.

LITERATURE CITED

Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. 2003. Bone loss and bone size after menopause. N Engl J Med 349:327–334.

Akazawa T, Muhesen S, editors. 2002. Neanderthal burials: excavations of the Dederiyeh Cave, Afrin, Syria. Studies in honor of Hisashi Suzuki. Kyoto: International Research Center for Japanese Studies.

Akkus O, Adar F, Schaffler MB. 2004. Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone 34:443–453.

Alexander RM. 1977. Allometry of the limbs of antelopes (Bovidae). J Zool Lond 183:125–146.

Alexander RM. 1981. Factors of safety in the structure of mammals. Sci Prog 67:109–130.

Alexander RM. 1998. Symmorphosis and safety factors. In: Weibel ER, Taylor CR, Bolis L, editors. Principles of animal design: the optimization and symmorphosis debate. Cambridge: Cambridge University Press. p 28–35.

Alfredson H, Nordström P, Lorentzon R. 1996. Total and regional bone mass in female soccer players. Calcif Tissue Int 59:438–442.

Alfredson H, Nordström P, Lorentzon R. 1997. Bone mass in female volleyball players: a comparison of total and regional bone mass in female volleyball players and nonactive females. Calcif Tissue Int 60:338–342.

Alfredson H, Hedberg G, Bergström E, Nordström P, Lorentzon R. 1998a. High thigh muscle strength but not bone mass in young horseback-riding females. Calcif Tissue Int 62:497–501.

Alfredson H, Nordström P, Pietilä T, Lorentzon R. 1998b. Long-term loading and regional bone mass of the arm in female volleyball players. Calcif Tissue Int 62:303–308.

Ankrom M, Patterson J, Patricia DA, Vetter U, Blackman M, Sponsellers P, Tayback M, Robey P, Shapiro J, Fedarko N. 1998. Age-related changes in human oestrogen receptor function and levels in osteoblasts. Biochem J 333:787–794.

Bailey AJ, Knott L. 1999. Molecular changes in bone collagen in osteoporosis and osteoarthritis in the elderly. Exp Gerentol 34:337–351.

Bailey AJ, Sims TJ. Ebbesen, EN, Mansell JP, Thomsen JS, Moskilde L. 1999. Age-related changes in the biochemical properties of human cancellous bone collagen: relationship to bone strength. Calcif Tissue Int 65:203–210.

Bakker AD, Soejima K, Klein-Nulend J, Burger EH. 2001. The production of nitric oxide and prostaglandin E(2) by primary bone cells is shear stress dependent. J Biomech. 34:671–677.

Bakker AD, Joldersma M, Klein-Nulend J, Burger EH. 2003. Interactive effects of PTH and mechanical stress on nitric oxide and PGE2 production by primary mouse osteoblastic cells. Am J Physiol Endocrinol Metab 285:608–613.

Baldwin KM, White TP, Arnaud SB, Edgerton VR, Kraemer WJ, Kram R, Raab-Cullen D, Snow CM. 1996. Musculoskeletal adaptations to weightlessness and development of effective countermeasures. Med Sci Sports Exerc 28:1247–1253.

Banse X, Sims TJ, Bailey AJ. 2002. Mechanical properties of adult vertebral cancellous bone: correlation with collagen intermolecular cross-links. J Bone Miner Res 17:1621–1628.

Barnes GL, Kostenuik PJ, Gerstenfeld LC, Einhorn TA. 1999. Growth factor regulation of fracture repair. J Bone Miner Res 14:1805–1815.

Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A, Seeman E. 1998. Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. J Bone Miner Res 13:500–507.

Bass SL, Saxon L, Daly RM, Turner CH, and Robling AG, Seeman E, Stuckey S. 2002. The effect of mechanical loading on the size and shape of bone in pre-, peri, and postpubertal girls: a study in tennis players. J Bone Miner Res 17:2274–2280.

Bätge B, Feydt A, Gebken J, Klein H, Notbohm H, Muller PK, Brinckmann J. 2000. Age-related differences in the expression of receptors for TGF-β in human osteoblast-like cells in vitro. Exp Clin Endocrinol Diabetes 108:311–315.

Ben-Itzhak S, Smith P, Bloom RA. 1988. Radiographic study of the humerus in Neandertals and *Homo sapiens sapiens*. Am J Phys Anthropol 77:231–242.

Ben-Porath I, Weinberg RA. 2004. When cells get stressed: an integrative view of cellular senescence. J Clin Invest 113:8–13.

Bennell KL, Malcolm SA, Khan KM, Thomas SA, Reid SJ, Brukner PD, Ebeling PR, Wark JD. 1997. Bone mass and bone turnover in power athletes, endurance athletes, and controls: a 12-month longitudinal study. Bone 20:477–484.

Bennike P, Bohr H. 1990. Bone mineral content in the past and present. Osteoporosis. 1990. p 89–91.

Bergman RJ, Grazit D, Kahn AJ, Gruber H, McDougall S, Hahn TJ. 1996. Age-related change in osteogenic stem cells in mice. J Bone Miner Res 11:568–577.

Bertram JEA, Biewener AA. 1988. Bone curvature: sacrificing strength for load predictability? J Theor Biol 131:75–92.

Bertram JEA, Swartz SM. 1991. The law of bone transformation: a case of crying Wolff? Biol Rev 66:245–273.

Biewener AA. 1991. Musculoskeletal design in relation to body size. J Biomech 24:19–29.

Biewener AA, Bertram JEA. 1993. Skeletal strain patterns in relation to exercise training during growth. J Exp Biol 185:51–69.

Biewener AA, Swartz SM, Bertram JEA. 1986. Bone modeling during growth: dynamic strain equilibrium in the chick tibiotarsus. Calcif Tissue Int 39:390–395.

Blanchet C, Giguère Y, Prud'homme D, Turcot-Lemay L, Dumont M, Leduc M, Côte S, Laflamme N, Rousseau F, Dodin S. 2003. Leisure physical activity is associated with quantitative ultra-

sound measurements independently of bone mineral density in postmenopausal women. Calcif Tissue Int 73:339–349.
Blimkie CJR, Rice S, Webber CE, Martin J, Levy D, Gordon CL. 1996. Effects of resistance training on bone mineral content and density in adolescent females. Can J Physiol Pharmacol 74:1025–1033.
Boaz T, Ciochon RL. 2004. Headstrong hominids. Nat Hist 113: 29–34.
Bonewald LF. 1999. Establishment and characterization of an osteocyte-like cell line, MLO-Y4. J Bone Miner Metab 17:61–65.
Boot AM, van der Sluis IM, de Muinck Keizer-Schrama SMPF, van Meurs JBJ, Krenning EP, Pols HAP, Uitterlinden AG. 2004. Estrogen receptor α gene polymorphisms and bone mineral density in healthy children and young adults. Calcif Tissue Int 74:495–500.
Bord S, Horner A, Beavan S, Compston J. 2001. Estrogen receptors alpha and beta are differentially expressed in developing human bone. J Clin Endocrinol Metab 86:2309–2314.
Bouvier H, Hylander WL. 1981. Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). J Morphol 167: 1–12.
Bouvier M, Hylander WL. 1996. The mechanical or metabolic function of secondary osteonal bone in the monkey *Macaca fascicularis*. Arch Oral Biol 41:941–950.
Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423:337–342.
Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E. 1998. Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. J Bone Miner Res 13:1814–1821.
Brahm H, Mallmin H, Michaëlsson K, Ström H, Ljunghall S. 1998. Relationships between bone mass measurements and lifetime physical activity in a Swedish population. Calcif Tissue Int 62:400–412.
Braidman IP, Hainey L, Batra G, Selby PL, Saunders PT, Hoyland JA. 2001. Localization of estrogen receptor beta protein expression in adult human bone. J Bone Miner Res 16:214–220.
Brooke-Wavell K, Jones PR, Hardman AE, Tsuritani I, Yamada Y. 2001. Commencing, continuing and stopping brisk walking: effects on bone mineral in postmenopausal women. Osteoporosis Int 12:581–587.
Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WJ. 1998. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268.
Burger EH, Klein-Nulen J. 1999. Responses of bone cells to biomechanical forces in vitro. Adv Dent Res 113:93–98.
Burr DB. 2002. Targeted and nontargeted remodeling. Bone 30: 2–4.
Burr DB, Martin RB, Schaffler MB, Radin EL. 1985. Bone remodeling in response to in vivo fatigue microdamage. J Biomech 18:189–200.
Burr DB, Ruff CB, Thompson DD. 1990. Patterns of skeletal histologic change through time: comparison of an Archaic Native American population with modern populations. Anat Rec 226:307–313.
Burr D, Robling A, Turner C. 2002. Effect of biomechanical stress on bones in animals. Bone 30:781–786.
Caler WE, Carter DR. 1989. Bone creep-fatigue damage accumulation. J Biomech 22:625–635.
Capozza RF, Cointry GR, Cure-Ramírez P, Ferretti JL, Cure-Cure C. 2004. A DXA study of muscle-bone relationships in the whole body and limbs of 2512 normal men and pre- and postmenopausal women. Bone 35:283–295.
Carlsson GE, Persson G. 1967. Morphological changes in the mandible after extraction and wearing of dentures: a longitudinal, clinical and x-ray cephalometric study covering 5 years. Odontol Rev 18:27–54.
Carroll SB, Grier J, Weatherbee S. 2001. From DNA to diversity: molecular genetics and the evolution of animal design. Oxford: Blackwell.
Carter DR, Beaupré GS. 2001. Skeletal function and form: mechanobiology of skeletal development, aging, and regeneration. New York: Cambridge University Press.
Carter DR, Caler WE. 1983. Cycle-dependent and time-dependent bone fracture with repeated loading. J Biomech Eng 105: 166–170.
Carter DR, Caler WE. 1985. A cumulative damage model for bone fracture. J Orthop Res 3:84–90.
Carter DR, Hayes WC. 1977a. Compact bone fatigue damage—a microscopic examination. Clin Orthop 127:265–274.
Carter DR, Hayes WC. 1977b. Compact bone fatigue damage. I. Residual strength and stiffness. J Biomech 10:323–337.
Carter DR, van der Meulen MC, Beaupré GS. 1996. Skeletal development: mechanical consequences of growth, aging, and disease. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego: Academic Press. p 333–350.
Cassell C, Benedict M, Specker B. 1996. Bone mineral density in elite 7- to 9-yr-old female gymnasts and swimmers. Med Sci Sports Exerc 28:1243–1246.
Chalmers J, Ray RD. 1962. The growth of transplanted foetal bones in different immunological environments. J Bone Joint Surg [Br] 44:149–164.
Chamay A, Tschantz P. 1972. Mechanical influences in bone remodeling. Experimental research on Wolff's law. J Biomech 5:173–180.
Chan GK, Duque G. 2002. Age-related bone loss: old bone, new facts. Gerontology 48:62–71.
Cheng B, Kato Y, Zhao S, Sprague E, Bonewald LF, Jiang JX. 2001. PGE(2) is essential for gap-junction communication between osteocyte-like MLO-Y4 cells in response to mechanical strain. Endocrinology 142:3464–3473.
Cherian PP, Cheng B, Gu S, Sprague E, Bonewald LF, Jiang JX. 2003. Effects of mechanical strain on the formation of gap junctions in osteocytes are mediated through the prostaglandin EP2 receptor. J Biol Chem 278:43146–43156.
Chiu CH, Hamrick MW. 2002. Evolution and development of the primate limb skeleton. Evol Anthropol 11:94–107.
Chow JW, Fox SW, Lean JM, Chambers TJ. 1998. Role of nitric oxide and prostaglandins in mechanically induced bone formation. J Bone Miner Res 13:1039–1044.
Churches AE, Howlett CR. 1981. The response of mature cortical bone to controlled time-varying loading. In: Cowin SC, editor. Mechanical properties of bone. New York: American Society of Mechanical Engineers. p 69–80.
Churchill SE, Formicola V. 1997. A case of marked bilateral asymmetry in the upper limbs of an Upper Palaeolithic male from Barma Grande (Liguria), Italy. Int J Osteoarchaeol 7:18–38.
Civitelli R. 1995. Cell-cell communication in bone. Calcif Tissue Int [Suppl] 56:29–31.
Clegg M, Aiello LC. 1999. A comparison of the Narikotome *Homo erectus* with juveniles from a modern human population. Am J Phys Anthropol 110:81–93.
Collet P, Uebelhart D, Vico L, Moro L, Hartmann D, Roth M, Alexandre C. 1997. Effects of 1- and 6-month spaceflight on bone mass and biochemistry in two humans. Bone 20:547–551.
Collins CJ, Sedivy JM. 2003. Involvement of the INK4a/Arf gene locus in senescence. Aging Cell 2:145–150.
Corruccini R, Nesbitt R, Ciochon R. 1992. Occlusofacial morphological change in relation to masticatory function. Am J Phys Anthropol [Suppl] 14:63–64 [abstract].
Cowin SC. 2001. Mechanics of materials. In: Cowin SC, editor. Bone biomechanics handbook, 2nd ed. Boca Raton: CRC Press. p 6-1–6-24.
Cowin SC, Moss ML. 2001. Mechanosensory mechanisms in bone. In: Cowin SC, editor. Bone biomechanics handbook, 2nd ed. Boca Raton: CRC Press. p 29-1–29-17.
Cowin SC, Moss-Salentijn L, Moss ML. 1991. Candidates for the mechanosensory system in bone. J Biomech Eng 113:191–197.
Cowin SC, Weinbaum S, Zeng Y. 1995. A case for bone canaliculi as the anatomical site of strain generated potentials. J Biomech 28:1281–1297.
Cui L, Ma YF, Yao W, Zhou H, Setterberg RB, Liang CT, Jee WS. 2001. Cancellous bone of aged rats maintains its capacity to

respond vigorously to the anabolic effects of prostaglandin E2 by modeling-dependent bone gain. J Bone Miner Metab 19:29–37.
Currey JD. 1959. Differences in the tensile strength of bone of different histological types. J Anat 93:87–95.
Currey J. 1984. The mechanical adaptations of bones. Princeton: Princeton University Press.
Currey JD. 2002. Bones: structure and mechanics. Princeton: Princeton University Press.
Currey JD, Alexander RM. 1985. The thickness of the walls of tubular bones. J Zool Lond [A] 206:453–468.
Dalén N, Olsson KE. 1974. Bone mineral content and physical activity. Acta Orthop Scand 45:170–174.
Daly RM, Saxon L, Turner CH, Robling AG, Bass SL. 2004. The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 34:281–287.
Davidson RM, Lingenbrink PA, Norton LA. 1996. Continuous mechanical loading alters properties of mechanosensitive channels in G292 osteoblastic cells. Calcif Tissue Int 59:500–504.
Day MH. 1971. Postcranial remains of *Homo erectus* from Bed IV, Olduvai Gorge, Tanzania. Nature 232:383–387.
Demes B. 1985. Biomechanics of the primate skull base. Adv Anat Embryol Cell Biol 94:1–59.
Demes B, Jungers WL, Selpin K. 1991. Body size, locomotion, and long bone cross-sectional geometry in Indriid primates. Am J Phys Anthropol 86:537–547.
Demes B, Stern JT Jr, Hausman MR, Larson SG, McLeod KJ, Rubin CT. 1998. Patterns of strain in the macaque ulna during functional activity. Am J Phys Anthropol 106:87–100.
Demes B, Qin Y-X, Stern JTJ, Larson SG, Rubin CT. 2001. Patterns of strain in the macaque tibia during functional activity. Am J Phys Anthropol 116:257–265.
Dodd JS, Raleigh JA, Gross TS. 1999. Osteocyte hypoxia: a novel mechanotransduction pathway. Am J Physiol (Cell Physiol) 48:598–C602.
Donahue SW, Jacobs CR, Donahue HJ. 2001. Flow-induced calcium oscillations in rat osteoblasts are age, loading frequency, and shear stress dependent. Am J Physiol Cell Physiol 281: 1635–1641.
Ducy P, Starbuck M, Priemel M, Shen J, Pinero G, Geoffroy V, Amling M, Karsenty G. 1999. A Cbfa1-dependent genetic pathway controls bone formation beyond embryonic development. Genes Dev 13:1025–1036.
Ducy P, Schinke T, Karsenty G. 2000. The osteoblast: a sophisticated fibroblast under central surveillance. Science 289:1501–1504.
Duncan RL, Turner CH. 1995. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int 57:344–358.
Düppe H, Cooper C, Gärdsell P, Johnell O. 1997. The relationship between childhood growth, bone mass, and muscle strength in male and female adolescents. Calcif Tissue Int 60:405–409.
Ehrlich PJ, Lanyon LE. 2002. Mechanical strain and bone cell function: a review. Osteoporosis Int 13:688–700.
Endo B, Kimura T. 1970. Postcranial skeleton of the Amud man. In: Suzuki H, Takai F, editors. The Amud man and his cave site. Tokyo: Academic Press of Japan. p 231–406.
Erdmann J, Kogler C, Diel I, Ziegler R, Pfeilschifter J. 1999. Age-associated changes in the stimulatory effect of transforming growth factor beta on human osteogenic colony formation. Mech Ageing Dev 110:73–85.
Ericksen MR. 1976. Cortical bone loss with age in three Native American populations. Am J Phys Anthropol 45:443–452.
Fajardo RJ, Müller R. 2001. Three-dimensional analysis of nonhuman primate trabecular architecture using microcomputed tomography. Am J Physl Anthropol 115:327–336.
Fehling PC, Alekel L, Clasey J, Rector A, Stillman RJ. 1995. A comparison of bone mineral densities among female athletes in impact loading and active loading sports. Bone 17:205–210.
Forwood MR. 1996. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. J Bone Miner Res 11:1688–1693.
Forwood MR, Burr DB. 1993. Physical activity and bone mass: exercises in futility? Bone Miner 21:89–112.
Frank JD, Ryan M, Kalscheur VL, Ruaux-Mason CP, Hozak RR, Muir P. 2002. Aging and accumulation of microdamage in canine bone. Bone 30:201–206.
French SA, Fulkerson JA, Story M. 2000. Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: a review of intervention trials. Prev Med 31:722–731.
Frisancho AR, Garn SM, Ascoli W. 1970. Subperiosteal and endosteal bone apposition during adolescence. Hum Biol 42:639–664.
Frost HM. 1964. Mathematical elements of lamellar bone remodelling. Springfield, IL: Charles C. Thomas.
Frost HM. 1973. Bone remodeling and its relation to metabolic bone disease. Springfield, IL: Charles C. Thomas.
Frost HM. 1979. A chondral theory of modeling. Calcif Tissue Int 28:181–200.
Frost HM. 1986. Intermediary organization of the skeleton. Boca Raton: CRC Press.
Frost HM. 1987. Bone "mass" and the "mechanostat": a proposal. Anat Rec 219:1–9.
Frost HM. 1990. Skeletal structural adaptations to mechanical usage (SATMU). Anat Rec 226:403–422.
Frost HM. 1994. Perspectives: a vital biomechanical model of synovial joint design. Antat Rec 240:1–18.
Frost HM. 1999. Joint anatomy, design, and arthroses: insights of the Utah paradigm. Anat Rec 255:162–174.
Fuchs RK, Bauer JJ, Snow CM. 2001. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. J Bone Miner Res 16:148–156.
Fujimura R, Noriko A, Watanabe M, Mukai N, Amagai H, Fukubayashi T, Hayashi K, Tokuyama K, Suzuki M. 1997. Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Miner Res 12:656–662.
Garn S. 1972. The earlier gain and later loss of cortical bone. Springfield, IL: Charles C. Thomas.
Garn SM, Rohmann CG, Wagner B. 1967. Bone loss as a general phenomenon in man. Fed Proc 26:1729–1736.
Garn SM, Guzman MA, Wagner B. 1969. Subperiosteal gain and endosteal loss in protein-calorie malnutrition. Am J Phys Anthropol 30:153–155.
Gasser JA. 1995. Assessing bone quantity by pQCT. Bone 17:145–154.
Goodship AE, Cunningham JL. 2001. Pathophysiology of functional adaptation of bone in remodeling and repair *in vivo*. In: Cowin SC, editor. Bone biomechanics handbook. Boca Raton: CRC Press. p 26-1–26-31.
Goodship AE, Lanyon LE, McFie H. 1979. Functional adaptation of bone to increased stress. J Bone Joint Surg Am 61:539–546.
Gonos E, Derventzi A, Kveiborg M, Agiostratidou G, Kassem M, Clark FCB, Jat SP, Rattan ISS. 1998. Cloning and identification of genes that associate with mammalian replicative senescence. Exper Cell Res 240:66–74.
Gould SJ, Lewontin RC. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc R Soc Lond [Biol] 205:581–598.
Greendale GA, Barrett-Connor E, Edelstein S, Sue I, Haile R. 1995. Lifetime leisure exercise and osteoporosis. Am J Epidemiol 141:951–959.
Groothausen J, Siemer H, Kemper HCG, Twisk J, Welten DC. 1997. Influence of peak strain on lumbar bone mineral density: an analysis of 15-year physical activity in young males and females. Pediatr Exerc Sci 9:159–173.
Gross TS, McLeod KJ, Rubin CT. 1992. Characterizing bone strain distributions in vivo using three triple rosette strain gauges. J Biomech 25:1081–1087.
Guggino SE, Lajeunesse D, Wagner JA, Snyder SH. 1989. Bone remodeling signaled by a dihydropyridine- and phenylalkylamine-sensitive calcium channel. Proc Natl Acad Sci USA 86:2957–2960.
Gurven M, Kaplan H. In press. Determinants of time allocation across the lifespan: a theoretical model and an application to the Machiguenga and Piro of Peru. Hum Nat.

Gustavsson A, Thorsen K, Nordström P. 2003. A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in healthy adolescent males. Calcif Tissue Int 73:108–114.

Haapasalo H. 1998. Physical activity and growing bone: development of peak bone mass with special reference to the effects of unilateral activity. Ann Chir Gynaecol 87:250–252.

Haapasalo H, Kannus P, Sievänen H, Pasanen M, Uusi-Rasi K, Heinonen A, Oja P, Vuori I. 1996a. Development of mass, density, and estimated mechanical characteristics of bones in Caucasian females. J Bone Miner Res 11:1751–1760.

Haapasalo H, Sievänen H, Kannus P, Heinonen A, Oja P, Vuori I. 1996b. Dimensions and estimated mechanical characteristics of the humerus after long-term tennis loading. J Bone Miner Res 11:864–872.

Haapasalo H, Kannus P, Sievänen H, Pasanen M, Uusi-Rasi K, Heinonen A, Oja P, Vuori I. 1998. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. J Bone Miner Res 13:310–319.

Haapasalo H, Kontulainen S, Sievänen H, Kannus P, Järvinen M, Vuori I. 2000. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 27:351–357.

Hall BK. 1999. Evolutionary developmental biology, 2nd ed. Dordrecht: Kluwer Academic Publishers.

Hamrick MW. 1999. A chondral modeling theory revisited. J Theor Biol 201:201–208.

Hamrick MW. 2003. Increased bone mineral density in the femora of GDF8 knockout mice. Anat Rec 272:388–391.

Hamrick MW, McPherron AC, Lovejoy CO. 2002. Bone mineral content and density in the humerus of adult myostatin-deficient mice. Calcif Tissue Int 71:63–68.

Hamrick MW, Pennington C, Byron CD. 2003. Bone architecture and disc degeneration in the lumbar spine of mice lacking GDF-8 (myostatin). J Orthop Res 21:1025–1032.

Harada S-I, Rodan GA. 2003. Control of osteoblast function and regulation of bone mass. Nature 423:349–355.

Hawkey DE, Merbs CF. 1995. Activity-induced musculoskeletal stress markers (MSM) and subsistence strategy changes among ancient Hudson Bay Eskimos. Int J Osteoarchaeol 5:324–338.

Heinonen A, Sievänen H, Kannus P, Oja P, Vuori I. 1996. Effects of unilateral strength training and detraining on bone mineral mass and estimated mechanical characteristics of the upper limb bones in young women. J Bone Miner Res 11:490–501.

Heinonen A, Oja P, Sievänen H, Pasanen M, Vuori I. 1998. Effect of two training regimes on bone mineral density in healthy perimenopausal women: a randomized controlled trial. J Bone Miner Res 13:483–490.

Heinonen A, Kannus P, Sievänen H, Pasanen M, Oja P, Vuori I. 1999. Good maintenance of high-impact activity-induced bone gain by voluntary, unsupervised exercises: An 8-month follow-up of a randomized controlled trial. J Bone Miner Res 14:125–128.

Heinonen A, Sievänen H, Kannus P, Oja P, Pasanen M, Vuori I. 2000. High-impact exercise and bones of growing girls: a 9-month controlled trial. Osteoporosis Int 11:1010–1017.

Heinonen A, Sievänen H, Kannus P, Oja P, Vuori I. 2002. Site-Specific skeletal response to long-term weight training seems to be attributable to principal loading modality: a pQCT study of female weightlifters. Calcif Tissue Int 70:469–474.

Heřt J, Přibylová E, Lišková M. 1972. Reaction of bone to mechanical stimuli. Part 3. Microstructure of compact bone of rabbit tibia after intermittent loading. Acta Anat 82:218–230.

Hildebrand M. 1985. Walking and running. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. Functional vertebrate morphology. Cambridge, MA: Belknap Press. p 38–57.

Hill K. 1993. Life history theory and evolutionary anthropology. Evol Anthropol 2:78–88.

Hlusko LJ, Suwa G, Kono RT, Mahaney MC. 2004. Genetics and the evolution of primate enamel thickness: a baboon model. Am J Phys Anthropol 124:223–233.

Holliday TW. 1997. Body proportions in Late Pleistocene Europe and modern human origins. J Hum Evol 32:423–447.

Holt BM, Ruff CB, Trinkaus E. 2004. The Wolff's law debate: throwing out the water, but keeping the baby. Am J Phys Anthropol [Suppl] 38:115–116 [abstract].

Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC, Cervera P, Le Bouc Y. 2003. IGF-a receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182–187.

Hoppa RD. 2000. What to do with long bones: toward a progressive palaeoauxology. Anthropologie (Brno) 38:23–32.

Hsieh Y-F, Turner C. 2001. Effects of loading frequency on mechanically induced bone formation. J Bone Miner Res 16:918–924.

Hsieh Y-F, Wang T, Turner C. 1999. Viscoelastic response of the rat loading model: implication for studies of strain-adaptive bone formation. Bone 25:379–382.

Hylander WL, Johnson KR. 1997. In vivo alveolar bone strain and mandibular functional morphology. Am J Phys Anthropol [Suppl] 24:134 [abstract].

Inoue K, Ohgushi H, Yoshikawa T, Okumura M, Sempuku T, Tamai S, Dohi Y. 1997. The effect of aging on bone formation in porous hydroxyapatite: biochemical and histological analysis. J Bone Miner Res 12:989–994.

Israel H. 1973. Age factor and the pattern of change in craniofacial structures. Am J Phys Anthropol 39:111–128.

Iuliano-Burns S, Saxon L, Naughton G, Gibbons K, Bass SL. 2003. Regional specificity of exercise and calcium during skeletal growth in girls: a randomized controlled trial. J Bone Miner Res 18:156–162.

Jacobs CR, Yellowley CE, Davis BR, Zhou Z, Cimbala JM, Donahue HJ. 1998. Differential effect of steady versus oscillating flow on bone cells. J Biomech 31:969–976.

Jaworski ZF, Liskova-Kiar M, Uhthoff HK. 1980. Effect of long-term immobilization on the pattern of bone loss in older dogs. J Bone Joint Surg [Br] 62:104–110.

Jee WS. 2001. Integrated bone tissue physiology: anatomy and physiology. In: Cowin SC, editor. Bone biomechanics handbook, 2nd ed. Boca Raton: CRC Press. p 1–68.

Jee WS, Li XJ, Schaffler MB. 1991. Adaptation of diaphyseal structure with aging and increased mechanical usage in the adult rat: a histomorphometrical and biochemical study. Anat Rec 230:332–338.

Johannsen N, Brinkley T, Englert V, Neiderauer G, Specker B. 2003. Bone response to jumping is site-specific in children: a randomized trial. Bone 33:533–539.

Jones DB, Bingmann D. 1991. How do osteoblasts respond to mechanical stimulation? Cells Mater 1:329–334.

Jones HN, Priest JD, Hayes WC, Tichenor CC, Nagel DA. 1977. Humeral hypertrophy in response to exercise. J Bone Joint Surg [Am] 59:204–208.

Jowsley J. 1960. Age changes in human bone. Clin Orthop 17: 210–217.

Jurmain R. 1999. Stories from the skeleton: behavioral reconstruction in human osteology. Amsterdam: Gordon and Breach Publishers.

Justesen J, Stenderup K, Eriksen EF, Kassem M. 2002. Maintenance of osteoblastic and adipocytic differentiation potential with age and osteoporosis in human marrow stromal cell cultures. Calcif Tissue Int 71:36–44.

Kannus P, Haapasalo H, Sievänen H, Oja P, Vuori I. 1994. The site-specific effects of long-term unilateral activity on bone mineral density and content. Bone 15:279–284.

Kannus P, Haapasalo H, Sankelo M, Sievänen H, Pasanen M, Heinonen A, Oja P, Vuori I. 1995. Effect of starting-age of physical activity on bone mass in the dominant arm of tennis and squash players. Ann Intern Med 123:27–31.

Kardinaal AFM, Hoorneman G, Väänänen K, Charles P, Ando S, Maggiolini M, Charzewska J, Rotily M, Deloraine A, Heikkinen J, Juvin R, Schaafsma G. 2000. Determinants of bone mass and bone geometry in adolescent and young adult women. Calcif Tissue Int 66:81–89.

Karlsson MK. 2001. Skeletal effects of exercise in men. Calcif Tissue Int 69:196–199.

Karlsson MK, Johnell O, Obrant KJ. 1993. Bone mineral density in weight lifters. Calcif Tissue Int 52:212–215.
Karlsson MK, Obrant KJ, Johnell O. 1995. Is bone mineral density advantage maintained long-term in previous weight lifters? Calcif Tissue Int 57:325–328.
Karlsson M, Bass S, Seeman E. 2001. The evidence that exercise during growth or adulthood reduces the risk of fragility fractures is weak. Best Pract Res Clin Rheumatol 15:429–450.
Karlsson KM, Karlsson C, Ahlborg HG, Valdimarsson O, Ljunghall S, Obrant KJ. 2003. Bone turnover responses to changed physical activity. Calcif Tissue Int 72:675–680.
Karsenty G. 1998. Genetics of skeletogenesis. Dev Genet 22:301–313.
Karsenty G. 1999. The genetic transformation of bone biology. Genes Dev 13:3037–3051.
Karsenty G. 2003. The complexities of skeletal biology. Nature 423:316–318.
Kato Y, Boskey A, Spevak L, Dallas M, Hori M, Bonewald LF. 2001. Establishment of an osteoid and preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. J Bone Miner Res 16:1622–1633.
Kerr D, Morton A, Dick I, Prince R. 1996. Exercise effects on bone mass in postmenopausal women are site-specific and load-dependent. J Bone Miner Res 11:218–225.
Khan KM, Bennell KL, Hopper JL, Flicker L, Nowson CA, Sherwin AJ, Crichton KJ, Harcourt PR, Wark JD. 1998. Self-reported ballet classes undertaken at age 10–12 years and hip bone mineral density in later life. Osteoporosis Int 8:165–173.
Khan K, McKay HA, Haapasalo H, Bennell KL, Forwood M, Kannus P, Wark JD. 2000. Does childhood and adolescence provide a unique opportunity for exercise to strengthen the skeleton? J Sci Med Sport 3:150–164.
Khan SN, Bostrom MP, Lane JM. 2000. Bone growth factors. Orthop Clin North Am 31:375–388.
Khosla S. 2001. Minireview: the OPG/RANKL/RANK system. Endocrinology 142:5050–5055.
Kimura T. 1974. Mechanical characteristics of human lower leg bones. J Faculty Sci Univ Tokyo Sect V Anthropol 4:319–393.
Knothe Tate ML. 2003. "Whiter flows the fluid in bone?" An osteocyte's perspective. J Biomech 36:1409–1424.
Kohrt WM. 2001. Aging and the osteogenic response to mechanical loading. Int J Sport Nutr Exerc Metab 11:137–142.
Kohrt WM, Snead DB, Slatopolsky E, Birge SJJ. 1995. Additive effects of weight-bearing exercise and estrogen on bone mineral density in older women. J Bone Miner Res 10:1303–1311.
Kontulainen S, Kannus P, Haapasalo H, Heinonen A, Sievänen H, Oja P, Vuori I. 1999. Changes in bone mineral content with decreased training in competitive young adult tennis players and controls: a prospective 4-year follow-up. Med Sci Sports Exerc 31:646–652.
Kontulainen S, Kannus P, Haapasalo H, Sievänen H, Pasanen MH, Oia P, Vuori I. 2001. Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Miner Res 16:195–201.
Kontulainen S, Sievänen H, Kannus P, Pasanen M, Vuori I. 2003. Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female raquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. J Bone Miner Res 18:352–359.
Kriska AM, Sandler RB, Cauley JA, LaPorte RE, Hom DL, Pambianco G. 1988. The assessment of historical physical activity and its relation to adult bone parameters. Am J Epidemiol 127:1053–1061.
Kronenberg HM. 2003. Developmental regulation of the growth plate. Nature 423:332–336.
Krovitz GE. 2003. Shape and growth differences between Neandertals and modern humans: grounds for a species-level distinction? In: Thompson J, Krovitz G, Nelson A, editors. Patterns of growth and development in the genus *Homo*. Cambridge: Cambridge University Press. p 320–342.
Lam YM, Chen X, Marean CW, Frey C. 1998. Bone density and long bone representation in archaeological faunas: comparing results from CT and photon densitometry. J Archaeol Sci 25: 559–570.
Lam YM, Pearson OM, Marean CW, Chen X. 2003. Bone density studies in zooarchaeology. J Archaeol Sci 30:1701–1708.
Lampl M, Johnston FE. 1996. Problems in the aging of skeletal juveniles: perspectives from maturation assessments of living children. Am J Phys Anthropol 101:345–355.
Lanyon LE. 1982. Biomechanical properties of bone and response of bone to mechanical stimuli: functional strain as a controlling influence on bone modeling and remodeling behavior. In: Hall BK, editor. Bone. Boca Raton: CRC Press. p 79–108.
Lanyon LE, Rubin CT. 1984. Static vs. dynamic loads as an influence on bone remodelling. Biomechanics 17:897–905.
Lauder GV. 1996. The argument from design. In: Rose MR, Lauder GV, editors. Adaptation. San Diego: Academic Press. p 55–91.
Lazenby RA. 1990. Continuing periosteal apposition II: the significance of peak bone mass, strain equilibrium, and age-related differentials for mechanical compensation in human tubular bones. Am J Phys Anthropol 82:473–484.
Ledger M, Holtzhausen L-M, Constant D, Morris AG. 2000. Biomechanical beam analysis of long bones from a late 18th century slave cemetery in Cape Town, South Africa. Am J Phys Anthropol 112:207–216.
Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P, Karsenty G. 1997. Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. Nat Genet 16: 307–310.
Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L. 2003. Endocrinology: bone adaptation requires oestrogen receptor-α. Nature 424:389.
Lees TC, Staines A, Taylor D. 2002. Bone adaptation to load: microdamage as a stimulus for bone remodeling. J Anat 201: 437–446.
Liang CT, Barnes J, Seedor JG, Quartuccio HA, Bolander M, Jeffrey JJ, Rodan GA. 1992. Impaired bone activity in aged rats: alteration at the cellular and molecular levels. Bone 13: 435–441.
Lieberman DE. 1996. How and why humans grow thin skulls: experimental evidence for systemic cortical robusticity. Am J Phys Anthropol 101:217–236.
Lieberman DE. 1999. Homology and hominid phylogeny: problems and potential solutions. Evol Anthropol 7:142–151.
Lieberman DE, Crompton AW. 1998. Responses to stress: constraints on symmorphosis. In: Weibel E, Taylor CR, Bolis L, editors. Principles of animal design: the optimization and symmorphosis debate. Cambridge: Cambridge University Press. p 78–86.
Lieberman DE, Pearson OM. 2001. Trade-off between modeling and remodeling responses to loading in the mammalian limb. Bull Mus Comp Zool 156:269–282.
Lieberman DE, Devlin MJ, Pearson OM. 2001. Articular surface area responses to mechanical loading: effects of exercise, age, and skeletal location. Am J Phys Anthropol 116:266–277.
Lieberman DE, McBratney BM, Krovitz G. 2002. The evolution and development of cranial form in *Homo sapiens*. Proc Natl Acad Sci USA 99:1134–1139.
Lieberman DE, Pearson OM, Polk JD, Demes B, Crompton AW. 2003. Optimization of bone growth and remodeling in response to loading in tapered mammalian limbs. J Exp Biol 206:3125–3138.
Lieberman DE, Polk JD, Demes B. 2004a. Prediction long bone loading from cross-sectional geometry. Am J Phys Anthropol 123:156–171.
Lieberman DE, Krovitz K, McBratney-Owen B. 2004b. Testing hypotheses about tinkering in the fossil record: the case of the human skull. J Exp Zool B 302 B:284–301.
Lieberman JR, Daluiski A, Einhorn TA. 2002. The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg [Am] 84:1032–1044.
Long JR, Zhang YY, Liu PY, Liu YJ, Shen H, Dvornyk V, Zhao LJ, Deng HW. 2004. Association of estrogen receptor α and vitamin

D receptor gene polymorphisms with bone mineral density in Chinese males. Calcif Tissue Int 74:270–276.
Lovejoy CO, Trinkaus E. 1980. Strength and robusticity of the Neandertal tibia. Am J Phys Anthropol 53:465–470.
Lovejoy CO, Burnstein AH, Heiple KG. 1976. The biomechanical analysis of bone strength: a method and its application to platycnemia. Am J Phys Anthropol 44:489–506.
Lovejoy CO, Cohn MJ, White TD. 1999. Morphological analysis of the mammalian postcranium: a developmental perspective. Proc Natl Acad Sci USA 96:13247–13252.
Lovejoy CO, McCollum MA, Reno PL, Rosenman BA. 2003. Developmental biology and human evolution. Annu Rev Anthropol 32:85–109.
Macchiarelli R, Bondioli L, Galichon V, Tobias PV. 1999. Hip bone trabecular architecture shows uniquely distinctive locomotor behaviour in South African australopithecines. J Hum Evol 36:211–232.
MacKelvie KJ, McKay HA, Kban KM, Croker PRE. 2001. A school-based exercise intervention augments bone mineral accrual in early pubertal girls. J Pediatr 139:501–508.
MacKelvie KJ, Khan KM, McKay HA. 2002. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. Br J Sports Med 36:250–257.
MacKelvie KJ, Petit MA, Khan KM, Beck TJ, McKay HA. 2004. Bone mass and structure are enhanced following a 2-year randomized controlled trial of exercise in prepubertal boys. Bone 34:755–764.
Main RP, Biewener AA. 2004. Ontogenetic patterns of limb loading, in vivo bone strains, and growth in the goat radius. J Exp Biol 207:2577–2588.
Majeska RJ. 2001. Cell biology of bone. In: Cowin SC, editor. Bone mechanics handbook, 2nd ed. Boca Raton: CRC Press. p 2-1–2-24.
Majors AK, Boehm CA, Nitto H, Midura RJ, Muschler GF. 1997. Characterization of human bone marrow stromal cells with respect to osteoblastic differentiation. J Orthop Res 15:546–557.
Makhluf H, Mueller S, Mizuno S, Glowacki J. 2000. Age-related decline in osteoprotegerin expression by human bone marrow cells cultured in three-dimensional collagen sponges. Biochem Biophys Res Commun 268:669–672.
Malina RM. 2001a. Physical activity and fitness: pathways from childhood to adulthood. Am J Hum Biol 13:162–172.
Malina RM. 2001b. Tracking physical activity across the lifespan. Pres Counc Phys Fitness Sports Res Dig 3:1–8.
Marotti G. 1996. The structure of bone tissues and the cellular control of their deposition. Ital J Anat Embryol 101:25–79.
Marsh RL, Ellerby DJ, Carr JA, Henry HT, Buchanan CI. 2004. Partitioning the energetics of walking and running: swinging the limbs is expensive. Science 303:80–83.
Martin RB. 1995. A mathematical model for fatigue damage repair and stress fracture in osteonal bone. J Orthop Res 13: 309–316.
Martin RB. 2000. Toward a unifying theory of bone remodeling. Bone 26:1–6.
Martin RB. 2002. Is all cortical bone remodeling initiated by microdamage? Bone 30:8–13.
Martin RB, Burr DB. 1982. A hypothetical mechanism for the stimulation of osteonal remodelingby fatigue damage. J Biomech 15:137–139.
Martin RB, Burr DB. 1989. Structure, function, and adaptation of compact bone. New York: Raven Press.
Martin RB, Burr DB, Sharkey NA. 1998. Skeletal tissue mechanics. New York: Springer-Verlag, Inc.
Mashiba T, Turner CH, Hirano T, Forwood MR, Johnston CC Jr, Burr DB. 2001. Effects of suppressed bone turnover by bisphosphonates on microdamage accumulation and biochemical properties in clinically relevant skeletal sites in beagles. Bone 28: 524–531.
Mathieu N, Pirzio L, Freulet-Marrière MA, Desmaze C, Sabatier L. 2004. Telomeres and chromosomal instability. Cell Mol Life Sci 61:641–656.
Mayoux-Benhamou MA, Leyge JF, Roux C, Revel M. 1999. Cross-sectional study of weight-bearing activity on proximal femur bone mineral density. Calcif Tissue Int 64:179–183.
McKay HA, Petit MA, Khan KM, Schutz RW. 2000. Lifestyle determinants of bone mineral: a comparison between prepubertal Asian- and Caucasian-Canadian boys and girls. Calcif Tissue Int 66:320–324.
McLean W, Olsen BR. 2001. Mouse models of abnormal skeletal development and homeostasis. Trends Genet 17:38–43.
McMahon TA. 1984. Muscles, reflexes and locomotion. Princeton: Princeton University Press.
Mednikova M, Trinkaus E. 2001. Femoral midshaft diaphyseal cross-sectional geometry of the Sunghir 1 and 4 Gravettian human remains. Anthropologie (Brno) 39:103–109.
Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gunberg CM, Zizic TM, Hagberg JM, Pratley RE, Hurley BF. 1993. Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. J Appl Physiol 74:2478–2484.
Mickelsfield L, Rosenberg L, Cooper D, Hoffman M, Kalla A, Stander I, Lambert E. 2003. Bone mineral density and lifetime physical activity in South African women. Calcif Tissue Int 73:463–469.
Mishra S, Knothe Tate ML. 2003. Effect of lacunocanalicular architecture on hydraulic conductance in bone tissue: implications for bone health and evolution. Anat Rec [A] 273:752–762.
Molleson T, Cruse K, Mays S. 1998. Some sexually dimorphic features of the human juvenile skull and their value in sex determination in immature skeletal remains. J Archaeol Sci 25:719–728.
Mori S, Burr DB. 1993. Increased intracortical remodeling following fatigue damage. Bone 14:103–109.
Morris FL, Naughton GA, Gibbs JL, Carlson JS, Wark JD. 1997. Prospective ten-month exercise intervention in premenarcheal girls: positive effects on bone and lean mass. J Bone Miner Res 12:1453–1462.
Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR. 1997. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89:773–779.
Muschler GF, Nitto H, Boehm CA, Easley KA. 2001. Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. J Orthop Res 19:117–125.
Nagurka ML, Hayes WC. 1980. An interactive graphics package for calculating cross-sectional properties of complex shapes. J Biomech 13:59–64.
Nakamura I, Rodan GA, le Duong T. 2003. Regulatory mechanism of osteoclast activation. J Electron Microsc 52:527–533.
Nicolas V, Prewett A, Bettica P, Mohan S, Finkleman RD, Baylink DJ, Farley JR. 1994. Age-related decreases in insulin-like growth factor-I and transforming growth factor-β in femoral cortical bone from both men and women: implications for bone loss with aging. J Clin Endocrinol Metab 78:1011–1016.
Nilsson BE, Westlin NE. 1971. Bone density in athletes. Clin Orthop 77:179–182.
Nishida S, Endo N, Yamigiwa H, Tanizawa T, Takahashi H. 1999. Number of osteoprogenitor cells in human bone marrow markedly decreases after skeletal maturation. J Bone Miner Metab 17:171–177.
Noble BS, Stevens H, Loveridge N, Reeve J. 1997. Identification of apoptotic changes in osteocytes in normal and pathological human bone. Bone 20:273–282.
Nordström P, Lorentzon R. 1996. Site-specific bone mass differences of the lower extremities in 17-year-old ice hockey players. Calcif Tissue Int 59:443–448.
Nordström P, Nordström G, Thorsen K, Lorentzon R. 1996. Local bone mineral density, muscle strength, and exercise in adolescent boys: a comparative study of two groups in different muscle strength and exercise levels. Calcif Tissue Int 58:402–408.
Nordström P, Nordström G, Lorentzon R. 1997. Correlation of bone density to strength and physical activity in young men

with a low to moderate level of physical activity. Calcif Tissue Int 60:332–337.
Nordström P, Pettersson U, Lorentzon R. 1998. Type of physical activity, muscle strength, and pubertal stage as determinants of bone mineral density and bone area in adolescent boys. J Bone Mineral Res 13:1141–1148.
Novotny V, Streda A, Brandejsky P, Novotny A. 2001. Some x-rays and body development examinations of many times the world champion and the Olympic games winner—a long-distance runner at his 71 years of age. Anthropologie (Brno) 39: 69–74.
Olsen BR, Reginato AM, Wang W. 2000. Bone development. Annu Rev Cell Dev Biol 16:191–220.
Ortner DJ. 1975. Aging effects on osteon remodeling. Calcif Tissue Res 18:27–36.
Orwoll ES, Ferar JL, Oviatt SK, Huntington K, McClung MR. 1987. The effect of swimming exercise on bone mass. In: Christiansen C, Johansen JS, Riis BJ, editors. Osteoporosis 1987. Copenhagen: Osteopress. p 494–498.
Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. 1997. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771.
Owan I, Burr D, Turner C, Qiu J, Tu Y, Onyia J, Duncan R. 1997. Mechanotransduction in bone: osteoblasts are more responsive to fluid forces than mechanical strain. Am J Physiol 273:810–815.
Parfitt AM. 2002. Targeted and nontargeted bone remodeling: relationship to basic multicellular unit organization and progression. Bone 30:5–7.
Parkkari J, Natri A, Kannus P, Mänttäri A, Laukkanen R, Haapasalo H, Nenonen A, Pasanen M, Oja P, Vuori I. 2000. A controlled trial of the health benefits of regular walking on a golf course. Am J Med 109:102–108.
Pauwels F. 1974. Über die Bedeutung der Markhöhle für die mechanische Beanspruchung des Röhrenknochens. Z Anat Entwickl Gesch 145:81–85.
Pauwels F. 1980. Biomechanics of the locomotor apparatus. Berlin: Springer Verlag.
Pavalko F, Chen N, Turner C, Burr D, Atkinson S, Hsieh Y-F, Qiu J, Duncan R. 1998. Fluid shear-induced mechanical signalling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. Am J Physiol 275:1591–1601.
Pavalko F, Norvell SM, Burr D, Turner CH, Duncan R, Bidwell JP. 2003. A model for mechanotransduction in bone cells: the load-bearing mechanosomes. J Cell Biochem 88:104–112.
Pearson OM. 2000. Activity, climate, and postcranial robusticity: implications for modern human origins and scenarios of adaptive change. Curr Anthropol 41:569–607.
Pettersson U, Nordström P, Lorentzon R. 1999a. A comparison of bone mineral density and muscle strength in young male adults with different exercise level. Calcif Tissue Int 64:490–498.
Pettersson U, Stålnacke B-M, Ahlénius G-M, Henriksson-Larsén K, Lorentzon R. 1999b. Low bone mass density at multiple skeletal sites, including the appendicular skeleton in amenorrheic runners. Calcif Tissue Int 64:117–125.
Pettersson U, Alfredson H, Nordström P, Henriksson-Larsén K, Lorentzon R. 2000a. Bone mass in female cross-country skiers: relationship between muscle strength and different BMD sites. Calcif Tissue Int 67:199–206.
Pettersson U, Nordström P, Alfredson H, Henriksson-Larsén K, Lorentzon R. 2000b. Effect of high impact activity on bone mass and size in adolescent females: a comparative study between two different types of sports. Calcif Tissue Int 10:207–214.
Plochocki JH, Organ J. 2003. The effects of differential mechanical loading on articular surface area in miniature swine. Am J Phys Anthropol [Suppl] 36:169 [abstract].
Plochocki JH, Ward CV, Smith D. 2004. Mechanical regulation of tibiofemoral joint growth: a computational analysis. Am J Phys Anthropol [Suppl] 38:160–161 [abstract].
Pocock N, Eisman J, Sambook P, Guinn T, Yeates M, Freund J. 1987. Prediction of bone density from measurements of muscle strength and physical fitness. In: Christiansen C, Johansen JS, Riis BJ, editors. Osteoporosis 1987. Copenhagen: Osteopress. p 990–992.
Polk JD, Demes B, Jungers WL, Biknevicius AR, Heinrich RE, Runestad JA. 2000. A comparison of primate, carnivoran and rodent limb bone cross-sectional properties: are primates really unique? J Hum Evol 39:297–326.
Ponce de Leon MS, Zollikofer PE. 2001. Neanderthal cranial ontogeny and its implications for late hominid diversity. Nature 412:534–537.
Quarto R, Thomas D, Liang CT. 1995. Bone progenitor cell deficits and age-associated decline in bone repair capacity. Calcif Tissue Int 56:123–129.
Raab DM, Crenshaw TD, Kimmel DB, Smith EL. 1991. A histomorphometric study of cortical bone activity during increased weight-bearing exercise. J Bone Miner Res 6:741–749.
Rausch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. 2004. The "muscle-bone unit" during the pubertal growth spurt. Bone 34:771–775.
Richmond BG, Nakatsukasa M, Ketcham R, Hirakawa T. 2004. Trabecular bone structure in human and chimpanzee knee joints. Am J Phys Anthropol [Suppl] 38:167 [abstract].
Riggs CM, Lanyon LE, Boyde A. 1993a. Functional associations between collagen fibre orientation and locomotor strain direction in cortical bone of the equine radius. Anat Embryol (Berl) 187:231–238.
Riggs CM, Vaughan LC, Evans GP, Lanyon LE, Boyde A. 1993b. Mechanical implications of collagen fibre orientation in cortical bone of the equine radius. Anat Embryol (Berl) 187:239–248.
Robling A, Burr D, Turner C. 2000. Partitioning a daily mechanical stimulus into discrete loading bouts improves the osteogenic response to loading. J Bone Miner Res 15:1596–1602.
Robling A, Burr D, Turner C. 2001. Recovery periods restore mechanosensitivity to dynamically loaded bone. J Exp Biol 204:3389–3399.
Robling A, Hinant F, Burr D, Turner C. 2002. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. J Bone Miner Res 17:1545–1554.
Rong H, Berg U, Tørring O, Sundberg CJ, Granberg B, Bucht E. 1997. Effect of acute endurance and strength exercise on circulating calcium-regulating hormones and bone markers in young healthy males. Scand J Med Sci Sports 7:152–159.
Rose M. 1991. The evolutionary biology of aging. Oxford: Oxford University Press.
Ross CF. 2001. In vivo function of the craniofacial haft: The interorbital "pillar." Am J Phys Anthropol 116:108–139.
Roux W. 1881. Der zuchtende Kampf der Teile, oder die "Teilauslese" im Organismus (Theorie der "funktionellen Anpassung"). Leipzig: Wilhelm Engelmann (cited in Martin and Burr, 1998).
Rubin CT. 1984. Skeletal strain and the functional significance of bone architecture. Calcif Tissue Int 36:11–18.
Rubin CT, Lanyon LE. 1984. Regulation of bone formation by applied dynamic loads. J Bone Joint Surg [Am] 66:397–402.
Rubin CT, Lanyon LE. 1985. Regulation of bone mass by mechanical strain magnitude. Calcif Tissue Int 37:411–417.
Rubin CT, McLeod KJ. 1996. Inhibition of osteopenia by biophysical intervention. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego: Academic Press. p 351–371.
Rubin C, Turner AS, Bain S, Mallinckrodt C, MacLeod K. 2001. Low mechanical signals strngthen long bones. Nature 412:603–604.
Rubin C, Turner A, Muller R, Mittra E, McLeod K, Lin W, Qin Y. 2002. Quantity and quality of trabecular bone in the femur are enhanced by a strong anabolic, noninvasive mechanical intervention. J Bone Miner Res 17:349–357.
Ruff CB. 2000a. Body size, body shape, and long bone strength in modern humans. J Hum Evol 38:269–290.
Ruff CB. 2000b. Biomechanical analyses of archaeological human skeletons. In: Katzenberg MA, Saunders SR, editors. Biological anthropology of the human skeleton. New York: Wiley-Liss. p 71–102.
Ruff C. 2002. Long bone articular and diaphyseal structure in Old World monkeys and apes. I: locomotor effects. Am J Phys Anthropol 119:305–342.

Ruff C. 2003a. Ontogenetic adaptation to bipedalism: age changes in femoral to humeral length and strength proportions in humans, with a comparison to baboons. J Hum Evol 45:317–349.
Ruff C. 2003b. Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. Bone 33:317–329.
Ruff CB, Trinkaus E, Walker A, Larsen CS. 1993. Postcranial robusticity in *Homo*. I: temporal trends and mechanical interpretation. Am J Phys Anthropol 91:21–53.
Ruff CB, Walker A, Trinkaus E. 1994. Postcranial robusticity in *Homo*. III: ontogeny. Am J Phys Anthropol 93:35–54.
Ruff CB, McHenry HM, Thackeray JF. 1999. Cross-sectional geometry of the SK 82 and 97 proximal femora. Am J Phys Anthropol 109:509–521.
Ruff CB, Trinkaus E, Holt B. 2000. Lifeway changes as shown by postcranial skeletal robustness. Am J Phys Anthropol [Suppl] 30:266 [abstract].
Ryan AS, Elahi D. 1998. Loss of bone mineral density in women athletes during aging. Calcif Tissue Int 63:287–292.
Ryan TM, Ketcham RA. 2002. The three-dimensional structure of trabecular bone in the femoral head of strepsirrhine primates. J Hum Evol 43:1–26.
Ryder KD, Duncan RL. 2000. Parathyroid hormone modulates the response of osteoblast-like cells to mechanical stimulation. Calcif Tissue Int 67:241–246.
Scariano JK, Simplicio SG, Montoya GD, Garry PJ, Baumgartner RN. 2004. Estrogen receptor β dinucleotide (CA) repeat polymorphism is significantly associated with bone mineral density in postmenopausal women. Calcif Tissue Int 74:501–508.
Scerpella TA, Davenport M, Morganti CM, Kanaley JA, Johnson LM. 2003. Dose related association of impact activity and bone mineral density in pre-pubertal girls. Calcif Tissue Int 72:24–31.
Schaffler M, Burr DB. 1984. Primate cortical bone microstructure: relationship to locomotion. Am J Phys Anthropol 65:191–197.
Schaffler MB, Burr DB. 1988. Stiffness of compact bone: effects of porosity and density. J Biomech 21:13–16.
Schaffler MB, Radin EL, Burr DB. 1989. Mechanical and morphological effects of strain rate on fatigue of compact bone. Bone 10:207–214.
Schaffler MB, Radin EL, Burr DB. 1990. Long-term fatigue behavior of compact bone at low strain magnitude and rate. Bone 11:321–326.
Scheuer L, Black S. 2000. Developmental juvenile osteology. London: Academic Press.
Sedlin ED, Frost HM. 1963. Variations in rate of human osteon formation. Can J Biochem Physiol 41:19–22.
Sedlin ED, Frost HM, Villanueva AR. 1963. Age changes in resorption in human rib cortex. J Gerontol 18:345–349.
Senut B. 1985. Computerized tomography of a Neandertal humerus from Le Régourdou (Dordogne, France): comparisons with modern man. J Hum Evol 14:717–723.
Sievänen H, Koskue V, Rauhio A, Kannus P, Heinonen A, Vuori I. 1998. Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. J Bone Miner Res 13:871–882.
Skerry T. 2001. Biomechanical Influences on skeletal growth and development. In: O'Higgins P, Cohn M, editors. Development growth and evolution. London: Academic Press. p 29–40.
Skerry TM, Lanyon LE. 1995. Interruption of disuse by short duration walking exercise does not prevent bone loss in the sheep calcaneus. Bone 16:269–274.
Skerry TM, Bitensky L, Chayen J, Lanyon LE. 1989. Early strain-related changes in enzyme activity in osteocytes following bone loading in vivo. J Bone Miner Res 4:783–788.
Slemenda CW, Johnston CC. 1993. High intensity activities in young women: site specific bone mass effects among female figure skaters. J Bone Miner Res 20:125–132.
Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CC Jr. 1991. Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 6:1227–1233.
Snow CM, Williams DP, LaRiviere J, Fuchs RK, Robinson TL. 2001. Bone gains and losses follow seasonal training and detraining in gymnasts. Calcif Tissue Int 69:7–12.
Söderman K, Bergström E, Lorentzon R, Alfredson H. 2000. Bone mass and muscle strength in young female soccer players. Calcif Tissue Int 67:297–303.
Söti C, Sreedhar AS, Csermely P. 2003. Apoptosis, necrosis and cellular senescence: chaperone occupancy as a potential switch. Aging Cell 2:39–45.
Specker B, Brinkley T. 2003. Randomized trial of physical activity and calcium supplementation on bone mineral content in 3- to 5-year-old children. J Bone Miner Res 18:885–892.
Stanford CM, Welsch F, Kastner N, Thomas G, Zaharias R, Holtman K, Brand RA. 2000. Primary human bone cultures from older patients do not respond at continuum levels of in vivo strain magnitudes. J Biomech 33:63–71.
Stock J, Pfeiffer S. 2001. Linking structural variability in long bone diaphyses to habitual behaviors: foragers from the southern African Later Stone Age and the Andaman Islands. Am J Phys Anthropol 115:337–348.
Stout SD. 1995. Intraskeletal variability in osteon population density. Am J Phys Anthropol [Suppl] 20:205–206 [abstract].
Stout SD, Paine RR. 1994. Brief communication: bone remodeling rates: a test of an algorithm for estimating missing osteons. Am J Phys Anthropol 93:123–129.
Sugimura T, Inada J, Sawa S, Kakudo Y. 1984. Dynamic responses of the skull caused by loss of occlusal force. J Osaka Dent Univ 18:29–42.
Sundberg M, Gärdsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I. 2001. Peripubertal moderate exercise increases bone mass in boys but not in girls: a population-based intervention study. Osteoporosis Int 12:230–238.
Sundberg M, Gärdsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I. 2002. Physical activity increases bone size in prepubertal boys and bone mass in prepubertal girls: a combined cross-sectional and 3-year longitudinal study. Calcif Tissue Int 71:406–415.
Szivek JA, Johnson EM, Magee FP. 1992. *In vivo* strain analysis of the greyhound femoral diaphysis. J Invest Surg 5:91–108.
Takano Y, Turner CH, Burr DB. 1996. Mineral anisotropy in mineralized tissues is similar among species and mineral growth occurs independently of collagen orientation in rats: results from acoustic velocity measurements. J Bone Miner Res 11:1292–1301.
Takeshita S, Namba N, Zhao JJ, Jiang Y, Genant HK, Silva MJ, Brodt MD, Helgason CD, Kalesnikoff J, Rauh MJ, Humphires RK, Krystal G, Teitelnaum SL, Ross FP. 2002. SHIP-deficient mice are severely osteoporotic due to increased numbers of hyper-resorptive osteoclasts. Nat Med 9:943–949.
Tanaka H, Liang CT. 1996. Mitogenic activity but not phenotype expression of rat osteoprogenitor cells in response to IGF-I is impaired in aged rats. Mech Ageing Dev 92:1–10.
Tang LY, Cullen DM, Yee JA, Jee WS, Kimmel DB. 1997. Prostaglandin E2 increases the skeletal response to mechanical loading. J Bone Miner Res 12:276–282.
Teegarden D, Proulx WR, Martin BR, Zhao J, McCabe GP, Lyle RM, Peacock M, Slemenda C, Johnston CC, Weaver CM. 1995. Peak bone mass in young women. J Bone Miner Res 10:711–715.
Teegarden D, Proulx WR, Kern M, Sedlock D, Weaver CM, Johnston CC, Lyle RM. 1996. Previous physical activity relates to bone mineral measures in young women. Med Sci Sports Exerc 28:105–113.
Teitelbaum SL, Tondravi MM, Ross FP. 1996. Osteoclast biology. In: Marcus R, Feldman D, Kelsey J, editors. Osteoporosis. San Diego: Academic Press. p 61–93.
Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizoneko PC, Bonjour J-P. 1992. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 75: 1060–1065.
Thomas T, Burguera B. 2002. Is leptin the link between fat and bone mass? J Bone Miner Res 17:1563–1569.

Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. 1999. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and inhibit differentiation to adipocytes. Endocrinology 140:1630–1638.
Thompson DD. 1980. Age changes in bone mineralization, cortical thickness, and Haversian canal area. Calcif Tissue Int 31:5–11.
Thompson JL, Nelson AJ. 2000. The place of Neandertals in the evolution of hominid patterns of growth and development. J Hum Evol 38:475–495.
Tillier A-M. 1999. Les enfants Moustériens de Qafzeh: interprétation phylogénétique et paléoauxologique. Paris: CNRS Éditions.
Tiziani V, Reichenberger E, Buzzo CS, Niazi S, Fukai N, Stiller M, Peters H, Salzano FM, Raposo do Amaral CM, Olsen BR. 1999. The gene for cherubism maps to chromosome 4p16. Am J Hum Genet 65:158–166.
Trinkaus E. 1976. The evolution of the hominid femoral diaphysis during the Upper Pleistocene in Europe and the Near East. Z Morphol Anthropol 67:291–319.
Trinkaus E, Ruff C. 1989. Diaphyseal cross-sectional morphology and biomechanics of the Fond-de-Foret 1 and the Spy 2 femur and tibia. Anthropol Prehist 100:33–42.
Trinkaus E, Ruff CB. 1996. Early modern human remains from eastern Asia: the Yamashita-cho 1 immature postcrania. J Hum Evol 30:299–314.
Trinkus E, Ruff CB. 1999a. Diaphyseal cross-sectional geometry of Near Eastern Middle Paleolithic humans: The femur. J Archaeol Sci 26:409–424.
Trinkus E, Ruff CB. 1999b. Diaphyseal cross-sectional geometry of Near Eastern Middle Paleolithic humans: The tibia. J Archaeol Sci 26:1289–1300.
Trinkaus E, Churchill SE, Villemeur I, Riley KG, Heller JA, Ruff CB. 1991. Robusticity versus shape: the functional interpretation of Neandertal appendicular morphology. J Anthropol Soc Nippon 99:257–278.
Trinkaus E, Churchill SE, Ruff CB. 1994. Postcranial robusticity in *Homo*. II: bilateral asymmetry and bone plasticity. Am J Phys Anthropol 93:1–34.
Trinkaus E, Churchill SE, Ruff CB, Vandermeersch B. 1999. Long bone shaft robusticity and body proportions of the Saint-Césaire 1 Châtelperronian Neanderthal. J Archaeol Sci 26: 753–773.
Tsuji T, Hughes FJ, McCulloch CAG, Melcher AH. 1990. Effects of donor age on osteogenic cells of rat bone marrow in vitro. Mech Ageing Dev 51:121–132.
Tsuzuku S, Ikegami Y, Yabe K. 1998. Effects of high-intensity resistance training on bone mineral density in young male powerlifters. Calcif Tissue Int 63:283–286.
Turner C. 1998a. Exercise as a therapy for osteoporosis: the drunk and the street lamp revisited. Bone 23:83–85.
Turner C. 1998b. Three rules for bone adaptation to mechanical stimuli. Bone 23:399–407.
Turner C. 1999. Site-specific skeletal effects of exercise: importance of interstitial fluid pressure. Bone 24:161–162.
Turner C, Forwood M, Otter M. 1994. Mechanotransduction in bone: do bone cells act as sensors of fluid flow? FASEB J 8:875–878.
Turner CH, Owan I, Takano Y. 1995a. Mechanotransduction in bone: role of strain rate. Am J Physiol 269:438–442.
Turner CH, Yoshikawa T, Forwood MR, Sun TC, Burr DB. 1995b. High frequency components of bone strain in dogs measured during various activities. J Biomech 39–44.
Turner CH, Takano Y, Owan I. 1995c. Aging changes mechanical loading thresholds for bone formation in rats. J Bone Miner Res 10:1544–1549.
Turner C, Robling A, Duncan R, Burr D. 2002. Do bone cells behave like a neuronal network? Calcif Tissue Int 70:435–442.
Turner RT, Riggs BL, Spelsberg TC. 1994. Skeletal effects of estrogen. Endocr Rev 15:275–300.
Ueki Y, Tiziani V,Santanna C, Maulik C, Garfinkle J, Ninomiya C, doAmaral C, Peters H, Habal M, Rhee-Morris L, Doss JB, Kreiborg S, Olsen BR, Reichenberger E. 2001. Mutations in the c-Abl-binding protein SH3BP2 cause excessive bone degradation in cherubism. Nat Genet 28:125–126.
Uhthoff HK, Jaworski ZF. 1978. Bone loss in response to long-term immobilisation. J Bone Joint Surg [Br] 60:420–429.
Ulrich CM, Georgiou CC, Gillis DE, Snow CM. 1999. Lifetime physical activity is associated with bone mineral density in premenopausal women. J Womens Health 8:365–375.
Umemura Y, Ishiko T, Yamauchi T, Kurono M, Mashiko S. 1997. Five jumps per day increase bone mass in rats. J Bone Miner Res 12:1480–1485.
Uusi-Rasi K, Haapasalo H, Kannus P, Pasanen M, Sievänen H, Oja P, Vuori I. 1997. Determinants of bone mineralization in 8 to 20 year old Finnish females. Eur J Clin Nutr 51:54–59.
Uusi-Rasi K, Sievänen H, Vuori I, Heinonen A, Kannus P, Pasanen M, Rinne M, Oja P. 1999. Long-term recreational gymnastics, estrogen use, and selected risk factors for osteoporotic fractures. J Bone Miner Res 14:1231–1238.
Valdimarsson O, Kristinsson JO, Stefansson SO, Valdimarsson S, Sigurdsson G. 1999. Lean mass and physical activity as predictors of bone mineral density in 16–20-year old women. J Intern Med 245:489–496.
van der Meulen MCH, Carter DR. 1995. Developmental mechanics determine long bone allometry. J Theor Biol 172:323–327.
van der Meulen MCH, Beaupré GS, Carter DR. 1993. Mechanobiologic influences in long bone cross-sectional growth. Bone 14:635–642.
van der Meulen MCH, Ashford MWJ, Kiratli BJ, Bachrach LK, Carter DR. 1996. Determinates of femoral geometry and structure during adolescent growth. J Orthop Res 14:22–29.
Van Langendonck L, Claessens AL, Vlietinck R, Derom C, Beunen G. 2003. Influence of weight-bearing exercise on bone acquisition in prepubertal monozygotic female twins: a randomized controlled prospective study. Calcif Tissue Int 72:666–674.
Van Wesenbeeck L, Odgren PR, Mackay CA, D'Angelo M, Safadi FF, Popoff SN, Van Hul W, Marks SC Jr. 2002. The osteoporotic mutation toothless (tl) is a loss-of-function frameshift mutation in the rat Csf1 gene: evidence of a crucial role for CSF-1 in osteoclastogenesis and endochondral ossification. Proc Natl Acad Sci USA 99:14303–14308.
Vashishth D. 2004. Rising crack-growth-resistance behavior in cortical bone: implications for toughness measurements. J Biomech 37:943–946.
Vashishth D, Behiri JC, Bonfield W. 1997. Crack growth resistance in cortical bone: concept of microcrack toughening, J Biomech 30:763–769.
Vashishth D, Verborgt O, Divine G, Fyhrie DP. 2000. Decline in osteocyte lacunar density is associated with accumulation of microcracks with age. Bone 26:375–380.
Verborgt O, Gibson GJ, Schaffler MB. 2000. Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue damage in vivo. J Bone Miner Res 15:60–67.
Vincentelli R, Grigorov M. 1985. The effect of Haversian remodeling on the tensile properties of human cortical bone. J Biomech 18:201–207.
Vuori I. 1996. Peak bone mass and physical activity: a short review. Nutr Rev 54:11–114.
Wainright SA, Biggs BA, Currey JD, Gosline JM. 1976. Mechanical design in organisms. Princeton: Princeton University Press.
Walker R, Hill K, Kaplan H, McMillan G. 2002. Age-dependency in hunting ability among the Ache of eastern Paraguay. J Hum Evol 42:639–657.
Weidenreich F. 1941. The extremity bones of *Sinanthropus pekinensis*. Paleontol Sin New Ser D 5:1–150.
Weinbaum S, Cowin SC, Zeng Z. 1994. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. J Biomech 27:339–360.
Weinert BT, Timiras PS. 2003. Invited review: theories of aging. J Appl Physiol 95:1706–1716.
Weiss E. 2003. Effects of rowing on humeral strength. Am J Phys Anthropol 121:293–302.
Welsh L, Rutherford OM, James I, Crowly C, Comer M, Wolman R. 1997. The acute effects of exercise on bone turnover. Int J Sports Med 18:247–251.

Welten DC, Kemper HCG, Post GB, van Mechelen W, Twisk J, Lips P, Teule GJ. 1994. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res 9:1089–1096.

West-Eberhard MJ. 2003. Developmental plasticity and evolution. New York: Oxford University Press.

Westerlind KC, Wronski TJ, Ritman EL, Luo Z-P, An K-N, Bell NH, Turner RT. 1997. Estrogen regulates the rate of bone turnover but bone balance in ovariectomized rats is modulated by prevailing mechanical strain. Proc Natl Acad Sci USA 94: 4199–4204.

Weyts FAA, Bosmans B, Niesing R, van Leeuwen JPTM, Weinans H. 2003. Mechanical control of human osteoblast apoptosis and proliferation in relation to differentiation. Calcif Tissue Int 72:505–512.

Whitfield JF. 2003. Primary cilium—is it an osteocyte's strain-sensing flowmeter? J Cell Biochem 89:233–237.

Wilkins AS. 2002. The evolution of developmental pathways. Sunderland, MA: Sinauer.

Windahl SH, Vidal O, Andersson G, Gustafsson JA, Ohlsson C. 1999. Increased cortical bone mineral content but unchanged bone mineral density in female ER$\beta^{-/-}$ mice. J Clin Invest 104:895–901.

Winter DA. 1990. Biomechanics and motor control of human movement, 2nd ed. New York: John Wiley & Sons.

Wittich A, Mautalen CA, Oliveri MB, Bagur A, Somoza F, Rotemberg E. 1998. Professional football (soccer) players have a markedly greater skeletal mineral content, density than age- and BMI-matched controls. Calcif Tissue Int 63:112–117.

Witzke KA, Snow CM. 2000. Effects of plyonetric jump training on bone mass in adolescent girls. Med Sci Sports Exerc 32: 1051–1057.

Woitge HW, Friendmann B, Suttner S, Farahmand I, Müller M, Schmidt-Gayak H, Baertsch P, Ziegler R, Seibel MJ. 1998. Changes in bone turnover induced by aerobic and anaerobic exercise in young males. J Bone Miner Res 13:1797–1804.

Wolff I, van Croonenborg JJ, Kemper HC, Kostense PJ, Twisk JW. 1999. The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre-and postmenopausal women. Osteoporosis Int 9:1–2.

Wolff J. 1986. The law of bone remodeling [translated from the 1892 original, Das Gesetz der Transformation der Knochen, by P. Maquet and R. Furlong]. Berlin: Springer Verlag.

Wolpoff MH. 1999. Paleoanthropology, 2nd ed. New York: McGraw-Hill.

Woo SLY, Kuei SC, Amiel D, Gomez MA, Hayes WC, White FC, Akeson WH. 1981. The effect of prolonged physical training on the properties of the long bone: a study of Wolff's law. J Bone Joint Surg [Am] 63:780–787.

Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. 1998. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 139:1329–1337.

You L, Cowin SC, Schaffler MB, Weinbaum S. 2001. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. J Biomech 34:1375–1386.

Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC. 1998. The effects of twelve weeks of bed rest on bone histology, biomechanical markers of bone turnover, and calcium homeostasis in eleven normal subjects. J Bone Miner Res 13:1594–1601.

Zhang D, Weinbaum S, Cowin SC. 1998. Electrical signal transmission in a bone cell network: the influence of a discrete gap junction. Ann Biomed Eng 26:644–659.

Zhao S, Zhang YK, Harris S, Ahuja SS, Bonewald LF. 2002. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. J Bone Miner Res 17:2068–2079.

Zilhão J, Trinkaus E, editors. 2002. Portrait of the artist as a child: the Gravettian human skeleton from the Abrigo do Lagar Velho—its archaeological context (Trabalhos de arqueologia 22). Lisbon: Instituto Protuguês.

Zioupos P. 2001. Ageing human bone: factors affecting its biomechanical properties and the role of collagen. J Biomater Appl 15:187–229.