PERSPECTIVE

JBMR

Forum on Aging and Skeletal Health: Summary of the Proceedings of an ASBMR Workshop

Sundeep Khosla, Teresita M Bellido, Marc K Drezner, Catherine M Gordon, Tamara B Harris, Douglas P Kiel, Barbara E Kream, Meryl S LeBoff, Jane B Lian, Charlotte A Peterson, Clifford J Rosen, John P Williams, Karen K Winer, and Sherry S Sherman

Author affiliations appear on p. 11

ABSTRACT

With the aging of the population, the scope of the problem of age-related bone loss and osteoporosis will continue to increase. As such, it is critical to obtain a better understanding of the factors determining the acquisition and loss of bone mass from childhood to senescence. While there have been significant advances in recent years in our understanding of both the basic biology of aging and a clinical definition of age-related frailty, few of these concepts in aging research have been evaluated adequately for their relevance and application to skeletal aging or fracture prevention. The March 2011 Forum on Aging and Skeletal Health, sponsored by the NIH and ASBMR, sought to bring together leaders in aging and bone research to enhance communications among diverse fields of study so as to accelerate the pace of scientific advances needed to reduce the burden of osteoporotic fractures. This report summarizes the major concepts presented at that meeting and in each area identifies key questions to help set the agenda for future research in skeletal aging.

KEY WORDS: AGING; GROWTH AND DEVELOPMENT; MENOPAUSE

Introduction

As noted by the Surgeon General's Report on Bone Health and Osteoporosis,[1] a substantial proportion of the elderly population will experience fractures associated with low bone mass: Ten million Americans 50 years of age and older already have osteoporosis by World Health Organization (WHO) criteria,[2] whereas 33 million more have osteopenia; the total with low bone mass could reach 61 million by 2020.[3] Likewise, the 2 million osteoporosis-related fractures in 2005 could exceed 3 million in 2025, with costs increasing from $16.9 billion to $25.3 billion annually.[4] Given the scope of the problem of age-related bone loss and osteoporosis, it is critical to obtain a better understanding of the factors determining the acquisition and loss of bone mass from childhood to senescence. In recent years, scientists have made dramatic advances in our understanding of the fundamentals of bone biology, as well as the basic biology of aging. However, these concepts, for the most part, have not been applied adequately to skeletal aging or fracture prevention by investigators either in the aging or the bone research communities. Thus the goal of the Forum on Aging and Skeletal Health, which was held in March 2011, was to bring together leaders in the fields of bone research and the biology of aging to exchange advances in their respective fields, cutting-edge concepts, and ideas for future directions. Such communication among diverse research communities and a bench-to-bedside translational focus was both timely and critical for advancing clinical and basic research on skeletal aging. Accelerating the pace of advances is urgently needed in order to develop better strategies to reduce the burden of osteoporotic fractures in the elderly. Thus the objectives of this workshop were to (1) summarize key state-of-the-science basic and clinical findings on the consequences of aging as well as the role of genetic, environmental, and lifestyle/behavioral factors on alterations in skeletal integrity and fracture risk, (2) identify gaps in knowledge and needs for improved methodology/technology/resources to facilitate research on healthy skeletal aging, and (3) identify opportunities for future research aimed at promoting skeletal health and reducing the risk of fractures across the lifespan. Those who contributed to this workshop through presentations and discussions are listed and acknowledged in the Appendix.

Received in original form July 6, 2011; accepted July 14, 2011. Published online September 2011.
Address correspondence to: Sundeep Khosla, MD, Endocrine Research Unit and Kogod Center on Aging, Mayo Clinic, 200 First Street SW, Guggenheim 7, Rochester, MN 55905, USA. E-mail: khosla.sundeep@mayo.edu or Sherry S Sherman, PhD, National Institute on Aging, Gateway Building, Suite 3C-307, 7201 Wisconsin Avenue, MSC 9205, Bethesda, MD 20892, USA. E-mail: shermans@nia.nih.gov

Journal of Bone and Mineral Research, Vol. 26, No. 11, November 2011, pp 1–14
DOI: 10.1002/jbmr.488

Bone Accretion and Loss: Influence of Nutrition and Physical Activity

Effects of puberty on bone structure and strength

Bone size and geometry change rapidly during puberty owing to the interplay of genetic, hormonal, nutritional, and mechanical factors. The skeleton undergoes marked changes in both length and width that are driven by the growth plate and the periosteum, respectively. Endochondral ossification is the process by which new tissue produced by the growth plate is turned into metaphyseal bone, a key step for bone elongation. Numerous local factors and systemic hormones regulate linear growth through their direct effects on the growth plate.

Bone growth in width occurs by periosteal apposition owing to the action of periosteal osteoblasts. This process determines cross-sectional bone size at the diaphysis and therefore is a crucial determinant of bone strength. During growth, osteoblasts on the periosteal surface continuously produce new bone in a process called *bone modeling*. This process is fundamentally different from the better-known *remodeling process*, whereby osteoblast and osteoclast action occurs on the same bone surface in a cyclic fashion.

Distal radius fractures are relatively common during early puberty. This may be due, in part, to the lag in cortical thickening at the radial metaphysis. As long as bone growth in length continues at a constant rate, metaphyseal bone is always newly built bone. Consequently, cortical thickness at the distal radial metaphysis changes little in prepubertal and early-pubertal children. During the same time, mechanical challenges to bone stability, in the event of a fall, increase markedly owing to increasing lever arms and body weight. This mismatch between increasing mechanical requirements and stagnant cortical thickness favors the development of fractures in the later phases of the growth period. Once growth in length slows down and eventually ceases in late puberty, cortical width increases rapidly, and the incidence of fractures at the distal radius decreases.[5]

In early pubertal girls, estradiol influences bone cross-sectional development by suppressing bone resorption at the endocortical surface.[6] Increases in volumetric bone density during puberty are associated with increases in trabecular thickening as a result of remodeling. There is no increase, however, in trabecular number throughout the growth period of childhood.

Mechanical forces play an essential role in directional regulation of bone growth, but there is presently very little information on how mechanical stimuli on the growth plate are converted into biologic signals that eventually determine the direction of growth in length.[7] Critical research gaps and questions identified were: (1) What are the directional regulators of longitudinal bone growth? (2) How is bone growth in width regulated? (3) How do hormonal changes during puberty interact with mechanical factors to change bone structure and strength? and (4) How do we analyze mechanical loads in a clinical setting?

Effects of physical activity on growing bone

The ideal exercise regimen to promote optimal lifetime bone health for children and adolescents is not known. Many of the studies in this area to date have used dual-energy X-ray absorptiometry (DXA) that likely underestimates the beneficial effects of exercise on a young skeleton.[8] DXA measures of growing children can be confounded by bone size and offer limited information on bone strength compared with other imaging modalities. A recent systematic review and meta-analysis evaluated randomized, controlled trials with interventions that were greater than 6 months in duration and examined bone strength.[9] The benefits of exercise were small and were noted only in pre- and early-pubertal boys. As expected, the benefits were linked with compliance. New skeletal assessment tools have emerged, such as peripheral quantitative computed tomography (pQCT) and high-resolution peripheral quantitative computed tomography (HRpQCT), that afford insights into how activity alters bone structure, geometry, microarchitecture, and strength. However, there has been a lack of agreement regarding which skeletal sites should be monitored in interventional studies of children, and this lack of standardization makes it difficult to pool data and reach consensus. Some of the studies to date also have not controlled for maturity, a critically important factor to consider because the bones of a child change substantially in shape, density, and strength at various stages of pubertal development. The type of exercise pursued and surface on which it is performed are also important considerations. Research gaps and questions identified were: (1) What exercise type and dose are most effective to elicit a positive bone-strength response in the growing skeleton? (2) What is the surface and site-specific adaptation of bone structure, geometry, and trabecular microstructure to exercise in growing boys and girls? and (3) At what maturational time point do optimal bone-strength adaptations to exercise occur in boys and girls?

Evaluation of bone in children with chronic illness

Chronic illness during growth and development poses threats to bone health by interfering with accrual and may lead to suboptimal peak bone mass. Both DXA and pQCT are valuable measures to identify deficiencies in bone mass in childhood that may arise owing to chronic illness. As noted earlier, DXA is a 2D projection technique that estimates areal bone mineral density (aBMD) and underestimates volumetric BMD (vBMD) in children with poor growth. DXA, however, remains the most widely used measure of skeletal health in all age groups. Guidelines and standards for clinical use of DXA in children are defined by the International Society for Clinical Densitometry (ISCD). The first ISCD Pediatric Position Development Conference was convened in 2007 and focused on issues related to bone density acquisition, interpretation, and reporting for children and adolescents. The first official position statement from the ISCD on pediatric densitometry was issued the following year and included recommendations for the prediction of fracture from DXA, the definition of low BMD, and the requirements for acceptable reference data.[10]

Crohn's disease is a chronic inflammatory bowel disease that is characterized by malnutrition, growth failure, and deficient bone accrual. Both the underlying inflammation and its required therapies (ie, glucocorticoids) are associated with bone and muscle deficits. pQCT provides measures of trabecular and

cortical vBMD, as well as valuable information on cortical dimensions.[11] Deficits in trabecular vBMD, cortical bone geometry, and muscle have been observed in Crohn's disease at the time of diagnosis. Tumor necrosis factor α (TNF-α) underlies the pathogenesis of intestinal inflammation and also inhibits bone formation. Investigational use of anti-TNF-α therapy in Crohn's disease is associated with significant increases in the bone-formation marker bone-specific alkaline phosphatase (BSAP) and increases in height *Z*-scores.[12]

Chronic illness may lead to delayed linear growth and sexual maturation, which result in inaccurate DXA measures owing to a systematic underestimation of vBMD in children who are small. Zemel and colleagues[13] recently provided methods for determining height-adjusted *Z*-scores for DXA measures in children who have short or tall stature for age. Several critical areas that need further investigation were identified: (1) Which noninvasive measures of bone health (eg, DXA, pQCT, or QCT) predict fracture in childhood chronic diseases? (2) Are trabecular and cortical bone deficits that develop during childhood chronic disease reversible? and (3) What are the indications for bisphosphonate therapy in childhood chronic diseases, and does therapy decrease short- and long-term fracture risk?

Effects of vitamin D on bone in children and mouse models

Humans and mice with impaired vitamin D action have hypocalcemia and secondary hyperparathyroidism, accompanied by hypophosphatemia. This results in osteomalacia and, in a growing skeleton, expansion of the cartilaginous growth plate (rickets).[14] In children with vitamin D receptor (VDR) mutations, intravenous administration of mineral ions (ie, phosphorus and calcium) leads to resolution of osteomalacia and rickets. Mouse models of VDR ablation were developed to determine which actions of the VDR are direct and which resulted from altered mineral homeostasis.[14] Experiments in *VDR* null mice have shown that rickets stems from impaired apoptosis of cells within the late hypertrophic chondrocyte region. Prevention of abnormal mineral ion levels leads to a normal skeleton in this model. Studies in a murine model of X-linked hypophosphatemia (associated with high serum fibroblast growth factor 23 [FGF-23] levels) and in mice with diets inducing hypercalcemia/hypophosphatemia demonstrated that low circulating phosphate levels were responsible for impaired hypertrophic chondrocyte apoptosis. Studies in cellular models demonstrated that phosphate induces hypertrophic, but not proliferative, chondrocyte apoptosis through a capase-9-dependent mitochondrial apoptotic pathway. Phosphate treatment of hypertrophic, but not proliferative, chondrocytes led to a decrease in mitochondrial membrane potential and Erk1/2 phosphorylation. Prevention of Erk1/2 phosphorylation inhibited hypertrophic chondrocytes apoptosis. Mice lacking Npt2a (a renal sodium-dependent phosphate transporter) also develop hypophosphatemia, but growth plate abnormalities resolve in association with increased 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] production.[15] However, *VDR/Npt2a* double knockout mice exhibit severe rickets. Therefore, receptor-dependent actions of $1,25(OH)_2D_3$ can compensate for hypophosphatemia and lead to normal growth plate development. Research gaps and questions identified were: (1) Within the growth plate, what is the mechanism of action of liganded VDR on growth? (2) What is the effect of hyperphosphatemic states on hypertrophic chondrocyte apoptosis, and how is the system modulated by vitamin D analogues and bisphosphonates? (3) What is the effect of the liganded VDR in the setting of hypophosphatemia on bone mineralization and biomechanical integrity? and (4) What is the role of FGF-23 in a growing and adult skeleton?

Effect of nutritional deprivation on bone

The childhood and adolescent years are critical for the achievement of peak bone mass. Growth, pubertal development, and bone accrual should occur simultaneously in a healthy adolescent and are nutrition-dependent physiologic processes. However, many common pediatric diseases present during these formative years, several of which are associated with malnutrition and compromised growth and bone accrual. The malnourished state is often associated with malabsorption of vitamin D and other key nutrients and underlying disease-related factors that can alter the patient's milieu and, ultimately, bone turnover. An overview of the long-term skeletal effects of malnutrition was presented in the context of the diseases, inflammatory bowel disease, cystic fibrosis, and anorexia nervosa. The potential roles of body weight and lean body mass, proresorptive cytokine secretion, and growth factors such as insulin-like growth factor 1 (IGF-1) were explored in each model. Anorexia nervosa, an extreme model of malnutrition-induced bone loss, includes hormonal abnormalities that alter both bone remodeling and bone marrow composition in this disease. In young women with this disorder, there is increased bone marrow fat that appears to result from preferential development of adipocytes over osteoblasts within bone marrow, potentially explaining the strikingly low bone-formation rates.[16] In addition, new data were presented from Hutchinson-Gilford Progeria syndrome, a pediatric model of early aging. Children with this rare, fatal genetic condition are emaciated in appearance but meet their caloric requirements for age and have normal bone turnover. Recent studies suggest that their skeletal phenotype is consistent with a skeletal dysplasia.[17] Research questions in this area include: (1) What are the effects of childhood malnutrition, as in anorexia nervosa, on peak bone mass and future osteoporosis risk? (2) What therapy is effective in preventing bone loss and/or promoting bone accretion in hypothalamic amenorrhea? (3) How important is peak bone mass in predicting skeletal outcomes in adulthood? (4) What is the relation among bone density, strength, and future osteoporosis risk? and (5) How can skeletal assessment tools be refined, including what data adjustments are most appropriate for evaluation of bone health in children?

Genetic and Other Risk Factors for Bone Loss and Fracture

Advances in bone genetics: BMD and fracture

The search for genes associated with a variety of skeletal traits has focused most recently on genome-wide association

studies (GWAS). This requires large study samples with well-phenotyped individuals and dense single-nucleotide polymorphism (SNP) genotyping. Using imputation to derive close to 2.5 million common variants, these studies can be combined using meta-analyses to provide the highest grade of evidence of association between genotypes and skeletal phenotypes. This has led to the creation of large consortia across the world that are studying musculoskeletal traits, including the Genetic Factors for Osteoporosis (GEFOS) and the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE). As the number of samples used in the meta-analyses has grown, the number of genome-wide significant associations has increased.[18–20] For the BMD phenotype, there is evidence that there will be a steep slope in the number of such significant findings discovered with each incremental addition to the sample sizes used, offering the promise that ultimately there may be better chances for prediction using genetic profiles as well as discovering new biologic pathways important to the skeleton. The most recent results from a meta-analysis of GWAS results from the GEFOS consortium demonstrate 34 genome-wide significant loci and 48 loci with suggestive genome-wide significance.

Early studies of fracture phenotypes using meta-analyses of candidate gene association studies have shown that variants in the *ESR1*, *Col1A1*, *VDR*, *and LRP5* genes are associated with either fractures in general or vertebral fractures in particular. The study of fracture phenotypes using GWAS has been less well developed, but several efforts are well underway. Using SNPs shown to be associated with BMD in the most recent GEFOS meta-analyses, a sample of 31,016 fracture cases and 102,444 controls was used to determine whether these SNPs also were associated with fractures. In 14 of the 96 SNPs that were tested, there was evidence of association with fractures, with odds ratios of up to 1.10 per SNP.

While considerable progress has been made in defining the genetics of bone density and fracture risk, additional work needs to focus on the following: (1) The findings from GWAS need to be expanded to determine what the causal SNPs and genes really are and to understanding the biologic mechanism(s) underlying the findings. (2) Since the current findings have explained a limited percent of the variance in skeletal traits studied to date, there is a need to discover more genetic variants explaining more of each of the traits. This will afford a better opportunity to add genetic factors to risk stratification (other types of genetic variation include rare variants that will require high-throughput sequencing, copy number variations (CNVs), DNA methylation patterns, and both gene-gene and gene-environment interactions). (3) Studies need to be made of genetic loci for bone phenotypes other than BMD and/or fracture. (4) The role of pleiotropic genetic effects needs to be examined. (5) There must be a deeper analysis of the heterogeneity in GWAS signals. (6) The role of genetics in the determination of peak bone mass and rates of bone loss (ie, perimenopausal, age-related) in future fracture risk must be examined. (7) The genetics of response-to-treatment requires attention. And (8) there should be a functional follow-up of novel genes/pathways.

Relation of race and ethnicity to fracture risk

Fracture incidence and risk factors for osteoporotic fractures (such as BMD) vary considerably among different race/ethnic groups. Differences among race/ethnic groups can result from biologic, behavioral, and cultural sources. There is also evidence of genetic admixture in all four major race/ethnic groups in the United States. As a result, identifying the reason for variability among race/ethnic groups can be complicated. Currently, 81% of those 65 years of age and older are non-Hispanic whites (NHWs), but this proportion is expected to decline to 60% by the year 2050 because significant increases in nonwhite groups—especially among Hispanic populations—are expected in the US population. Most fracture-related data from nonwhites focus on blacks, in whom rates of hip fracture, clinical vertebral fracture, and fracture of the upper and lower appendages are lower than in whites.[21] Smaller amounts of data suggest lower hip fracture rates in Hispanics and Asian Americans than in whites; limited data suggest that this also may be true at some other skeletal sites.[22,23] Fracture data for other race/ethnic groups are very sparse. Consistent with lower fracture rates, blacks have higher BMD values than whites, whereas Asians have lower fracture risk despite lower BMD values than whites at most skeletal sites.[24] The difference in BMD between whites and Hispanics depends on skeletal site. After adjustment for body size, inconsistencies between fracture risk and BMD between Asians and whites diminish.[25] Prospective data on age-related changes in bone mass are limited but suggest rates of loss that are similar or reduced in blacks and Asians compared with whites, depending on skeletal site and gender.[26] Areas for further research include (1) obtaining more data on fracture rates and age-related changes in bone mass in diverse populations, (2) identifying mechanisms that can explain the observed differences in fracture risk among different racial/ethnic groups, and (3) exploring other factors accounting for racial/ethnic differences in bone loss and fracture rates such as differences in endocrine patterns, body composition, bone geometry, and fall risk.

Impact of age-related changes in renal function on bone metabolism

About 30% of patients over the age of 65 with decreased BMD also have decreased estimated glomerular filtration rate (eGFR), and about 26 million Americans have decreased eGFR (stage 2 to 3 chronic kidney disease [CKD]), most with no history of actual kidney disease. Individuals with decreased BMD and eGFR may have either underlying kidney disease or simply age-related declines in kidney function and bone status without the presence of actual kidney disease per se. In the case of frank kidney disease, there is a well-recognized entity referred to as *chronic kidney disease–mineral bone disorder* (CKD-MBD). The underlying pathogenesis of this disorder involves the loss of skeletal anabolism secondary to the elaboration of inhibitors of the Wnt signaling pathway such as Dkk1. In fact, even early kidney disease induces the circulation of Wnt inhibitors before the appearance of elevated inorganic phosphate and parathyroid hormone (PTH). Also, early on, FGF-23 and sclerostin levels are increased, which results in decreases in $1,25(OH)_2D_3$

production and a decrease in bone-formation rate. The early onset of the CKD-MBD is reinforced by development of the classic pathophysiology of secondary hyperparathyroidism as the kidney disease progresses, leading to the 17-fold increase in hip fracture risk in patients with CKD-MBD.[27,28]

In contrast to the pathogenesis of CKD-MBD, the precise relation between low bone density and reduced eGFR in older individuals without frank kidney disease is less well understood. A nested case-control study from the Women's Health Initiative has demonstrated that serum cystatin C concentrations were associated with an increased risk for hip fracture.[29] In distinction to this study, data from a study of 427 postmenopausal women followed longitudinally for 25 years failed to demonstrate any additional value of adding GFR to the estimate of fracture risk using FRAX. These findings suggest that in older persons with decreased BMD and eGFR, the aging kidney is not directly related to the skeleton, but this is an area that needs more research. Areas identified for further research include (1) determining whether elderly patients with decreased BMD and eGFR have kidney disease, (2) identifying the direct effects of the aging kidney on the skeleton, (3) determining whether patients with the aging kidney have CKD-MBD (specifically, are Wnt inhibitors elevated? Is FGF-23 increased? Is sclerostin increased? Are leptin, serotonin, etc. affected?), (4) defining the effects of skeletal anabolic therapy on cardiovascular risk in elderly patients with decreased BMD and eGFR, (5) establishing consensus on the pathogenesis of CKD-MBD, (6) validating new biomarkers in CKD-MBD and clinical implementation of their use (eg, FGF-23, vascular calcium, DKK1, etc.), and (7) discovering new therapeutic approaches to CKD-MBD (all the approved pharmacologic agents are labeled for control of PTH levels, an off-target and late component of the syndrome).

Relation of vitamin D to falls in the elderly

In an early, large trial of institutionalized seniors in France treated with vitamin D and calcium, the risk for fracture was reduced within the first 6 months, suggesting that the intervention may have reduced falls. In observational studies addressing the relation between serum 25-hydroxyvitamin D [25(OH)D] concentrations and falls, there has been a clear increase in risk for falls when concentrations are below 25 nmol/L.[30] In later intervention studies, the effect of vitamin D and calcium on fractures and falls was less clear. Several meta-analyses of these studies on the effect of vitamin D on falls showed a significant decrease in fall incidence of between 5% and 20%. There was a suggestion that doses greater than 700 or 800 IU were required for the prevention of falls and that the very frail institutionalized population responded to a greater extent.[31] Recently, data from an Australian study suggested that very large doses administered infrequently (500,000 IU once per year) actually might have adverse effects on fall risk.[32]

The mechanism by which vitamin D status affects fall risk is not well understood, although some studies have demonstrated an association between serum concentrations of vitamin D and physical performance, strength, and balance.[33] Improvement in these domains in vitamin D intervention studies has not been clearly shown. Areas for further research include (1) defining the mechanism of how vitamin D might prevent falls, (2) defining a dose-response effect and whether higher doses prevent more falls as well as whether a calcium supplement is necessary, (3) determining whether falls are associated with polymorphisms of vitamin D–related genes, and (4) defining whether vitamin D prevents falls in the general population of older persons or only in the frail?

Frailty and falls as contributors to fracture

Falls are common in older individuals and are associated with high morbidity and mortality as well serious injuries such as traumatic fractures, which frequently require hospitalization. This public health problem is also costly—associated care costs in 2020 are expected to exceed $32 billion annually. Understanding the role of extraosseous factors in fractures is critical because traditionally assessed skeletal parameters of bone density, architecture, geometry, and other indicators of bone strength and quality offer useful but very incomplete information in predicting fractures in individuals.[34]

Research in fall prevention has produced significant advances over the past 20 years.[35] Numerous risk factors that cause or contribute to falls have been identified and include environmental hazards, muscle weakness, gait and balance disorders, functional impairment, impaired vision, memory loss, psychoactive medications, and prior falls. Clinical trials have led to successful strategies for reducing falls, the most promising of which include multifactorial fall risk assessments, targeted exercise, and mitigation of physiologic deficiencies (eg, vitamin D) and environmental hazards (eg, environmental modification, hip protectors, etc.).[36]

Falls are common, potentially debilitating, and expensive. They are, however, to a large extent preventable with existing technologies and application of promising fall risk-reduction strategies. Areas for further research include (1) identifying additional risk factors for serious injuries, impairment, and morbidity in the aging and frail population by elucidating the interaction of low bone mass and the propensity to fall that results in fractures, (2) evaluating causation versus association and modifiability of risk factors in the home and institutional settings, as well as in special populations, (3) developing more efficacious, cost-effective fall-prevention technologies and approaches, (4) developing approaches to improve surveillance and remediation of modifiable risk factors, and (5) developing strategies for translation of and increased adherence to efficacious fall-prevention opportunities among both community-dwelling and institutionalized older persons.

Treatment Approaches for Aging and Bone and Novel Skeletal Indicators of Longevity

Special considerations in treating osteoporosis in the elderly

Age is an independent risk factor for an osteoporotic fracture, and individuals in long-term care (LTC) facilities generally are in their 80s and 90s. Hip fractures are particularly devastating in this population and result in significant morbidity and mortality. Although most men and women in LTC facilities have low BMD,

few studies have determined who in these cohorts is at highest risk and thus who should be treated with antiosteoporosis drugs. Even more remarkable is the lack of randomized clinical trials for osteoporosis interventions in LTC facilities. There are several reasons for the paucity of trials in these cohorts, including the many comorbidities associated with inhabitants of LTC facilities, the lack of appropriate systems for collecting data, the reluctance of the pharmaceutical industry to participate, and the socioeconomic barriers inherent in consenting and conducting clinical trials outside a university setting. Thus the pertinent clinical questions and research gaps are (1) identifying who is at the greatest risk for fracture in LTC facilities, (2) determining the most cost-effective means of conducting osteoporosis trials in the frail elderly, (3) identifying how randomized, placebo-controlled clinical trials can be performed practically in LTC facilities, and (4) determining the optimal predictors and primary endpoints for these trials?

Skeletal indicators of longevity

There is increasing evidence that longevity can be prolonged by specific interventions in mice that include calorie restriction as well as treatment with rapamycin, an inhibitor of mTor, at 9 months of age.[37] Both these interventions share a common target, the IGF/IRS signaling pathway, which is also regulated by energy status and nutrient intake. Skeletal size increases in response to growth hormone, and it appears that IGF-1, which is increased by growth hormone, may be a surrogate marker for longevity, depending on the developmental context. For example, smaller, thinner cortices and reduced femoral length early in life (a phenotype often seen with low IGF-1 conditions) are associated with greater longevity in mice. In contrast, by midlife in rodents, thicker cortices, greater bone mass, and changes in memory T cells are tied to significantly longer life spans. Interestingly, there are similar data in humans that low bone mass is a marker of greater mortality. Thus the pertinent research questions going forward are: (1) Are there discrete skeletal surrogates that can define lifespan in rodents and humans? (2) What part of the IGF pathway targets the skeleton and defines longevity and by what mechanism? and (3) Are there other interventions timed at various stages of life that can be used to extend lifespan?

Aging-Related Changes in Bone Structure and Cellular Activity

Effects of body composition on bone and the muscle-bone unit in healthy youth

Bones change in size, shape, and spatial dimensions as they adapt to growth and functional demands. The skeleton adapts to loads with increases in size and bone mass in the periosteal envelope, as well as other changes in geometry.[38] The maximal bone mass in young adulthood is a reflection of increases in body size. Other factors that contribute to overall bone strength are gender, ethnicity, loading or unloading of bone, diseases, and genetics. Use of pQCT has provided important insights into the effects of gender and race on structural measures in growing children and adolescents. In females compared with males, cortical bone mineral content (BMC), periosteal diameter, and section modulus were lower at all Tanner stages. Blacks have higher measures of cortical bone than whites at Tanner stages I to IV, but the differences in cortical BMC, periosteal diameter, and section modulus were diminished by Tanner stage V. For both gender and race, adjustment for muscle cross-sectional area attenuated but did not eliminate the observed structural differences.[39] Hence gender and racial differences in bone strength result from differences in maturation, size, and body composition and not differences in the muscle-bone functional unit.

Bone strength is regulated by the mechanical loads on bone, and these loads generally arise from muscle forces rather than body weight or fat. Studies of overweight versus healthy-weight girls show that overweight girls have a greater bone area, density, and strength, but their bone strength is lower relative to their body weight and fat.[40] Muscle force can be increased by resistance training. Conversely, mechanical unloading in patients with muscle disorders is associated with reduced muscle mass and weaker bones.

Future studies are needed to address the following questions: (1) Does the muscle-bone unit adequately describe bone changes during growth? (2) Can the muscle-bone unit be optimized during growth or with nutritional (eg, vitamin D), exercise, or other interventions to protect against future fracture risk in later life? (3) Is there only one or multiple intervention windows of opportunity? (4) How does bone adapt to childhood factors in later adult life? and (5) What is the impact of increasing obesity on the muscle-bone unit and bone strength and lifetime fracture risk?

Changes in bone strength and skeletal loading with age

The exponential rise in fractures during aging is likely a consequence of reduced bone strength and possibly changes in skeletal loading. Bone strength depends on bone density and quality and is affected by bone geometry, architecture, and turnover. Studies of human cadaveric specimens show lower whole-bone strength in older than in younger adults. Imaging of bone structure in vivo (using QCT, pQCT, or HRpQCT) provides important information about age-related changes in bone architecture and estimates of bone strength. A population-based QCT study of the spine showed reductions in vertebral compressive strength with age that were greater in women than in men.[41] Femoral strength measures (in a sideways fall configuration) also showed age-related decreases at the hip, with larger reductions in women versus men; these differences exceeded those reported in femoral neck BMD.[42] The results of these and other studies indicate that whole-bone strength decreases markedly with age owing to reductions in trabecular and cortical bone density, decreases in cortical thickness, and marked increases in cortical porosity. The contributions of changes in cortical versus trabecular compartments, however, vary according to age, site (eg, spine, hip, and distal radius), and superimposed diseases. Resistance to fracture at the tissue level also is attenuated during aging, and there is evidence of greater

crack initiation and extension of cracks in aging bone. Currently, however, the role of age-related changes in tissue properties is not known.

Alterations in skeletal loading also may occur during aging, although more data are needed. Most hip fractures are associated with a fall, and fall propensity increases with age. Other factors that have an impact on hip fracture risk include fall force, soft tissue thickness, and muscle strength. Vertebral loading that may affect fractures of the spine varies greatly with types of daily activities, severity of spinal curvature, and muscle strength.

Research gaps and questions in this area include: (1) What is the relative contribution of age-related changes in bone tissue mechanical properties to bone strength? (2) What is the role of bone morphology and its heterogeneity in bone strength? (3) How well do changes in bone architecture and/or strength estimates predict fracture risk in prospective clinical studies? (4) How can deleterious age-related changes in bone structure be prevented? (5) Is it possible to define the effects of therapeutic interventions on cortical and trabecular compartments that might benefit individual patients? and (6) How do age-related changes in muscle strength and neuromuscular control influence skeletal loading owing to falls or activities of daily living?

Role of the osteocyte in mechanotransduction and in age-related bone loss

Recent evidence indicates that osteocytes located in lacunae in mineralized bone sense mechanical stimuli that activate or inactivate bone resorption with skeletal unloading or loading, respectively.[43,44] Osteocytes are multifunctional cells that comprise more than 90% to 95% of all bone cells in the adult skeleton. They survive for decades and have long dendritic processes and complex lacunocanalicular networks that are connected to the vascular system; the canaliculi also connect the lacunae to the bone surface. In response to mechanical strain, osteocytes signal through molecules that include calcium, prostaglandins, ATP, and nitric oxide; a major pathway involves Wnt/β-catenin. Research studies indicate that osteocytes participate in the following functions: (1) control of mineralization (they promote mineralization and bone formation [with expression of Phex and Dmp1] or inhibit mineralization and bone formation [with expression of sclerostin and MEPE/OF45]), (2) regulation of phosphate homeostasis and secretion of FGF-23, (3) calcium homeostasis, (4) regulation of osteoblast activity (sclerostin is a late osteocyte-selective factor), (5) recruitment of osteoclasts, in which osteocyte viability and cell death play a role, and (6) muscle myogenesis, potentially through the production of secreted factors that affect muscle cells (eg, C2C12 cells).[45] While bone adapts to strains and there is a loss of the anabolic response to skeletal loading during aging, this may be related to compromise of the osteocyte and/or its surrounding matrix. In summary, osteocytes are involved in bone remodeling and therefore may play an important role in skeletal aging. Since osteocytes also may regulate muscle cells through secreted factors, changes in osteocyte function also may be related to age-related loss of muscle mass. Future research questions include the following: (1) Are the specific genes activated by osteocyte secreted factors fully responsible for the accelerated myogenic programming seen in early C2C12 myoblasts? (2) Are the signaling pathways in C2C12 myotubes the same as in primary muscle cells? (3) What happens with age to the production of these osteocytic muscle-stimulating factors? (4) Can bones modulate skeletal muscle function? (5) Can bone-secreted factors be used to treat muscle diseases? (6) What is the role of the osteocyte in specific bone diseases? and (7) Can muscle-secreted factors affect bone?

Exercise and the preservation of bone health with aging

Clinical studies and multiple meta-analyses show that exercise can generate modest increases in BMD of 1% to 3% in adults.[46,47] Prospective cohort and case-controlled studies suggest that high levels of physical activity can reduce the risk of hip fractures by 30% to 40%, but there are no large randomized clinical trials of the effects of exercise on fracture incidence. Among studies that evaluated exercise exposure in a quantitative manner, the minimal levels of physical activity associated with a reduction in fracture risk included the following components: $\geq$9 to 14.9 MET-h/week of physical activity, $\geq$1290 kcal/week, or $\geq$3 to 4 hours of walking per week. Although recommendations for exercise generally include weight-bearing endurance activities three to five times per week and resistance exercises two to three times per week, some components of these guidelines are derived from general health recommendations. According to preclinical studies, exercise has more robust effects on bone strength than pharmacologic interventions, although the apparent fracture benefit from these preclinical studies is unproven in humans.

There is a common belief that some athletes have low BMD values because they participate in weight-supported (eg, cycling or swimming) rather than weight-bearing (eg, running or gymnastics) activities. However, the BMD values in some of these athletes may be below normal because under certain conditions exercise may cause bone loss. An exercise-induced reduction in serum calcium and increase in PTH and bone turnover biomarkers is one potential mechanism underlying bone loss in response to exercise.[48,49] Although both lean and fat tissue are directly associated with bone mass, the association is stronger for lean tissue. At present, however, clinical knowledge of the skeletal effects of exercise lags far behind preclinical knowledge. Clinical intervention trials are needed that test the mechanisms that have emerged from preclinical research. Unanswered questions and future areas of research include: (1) What is the best standardized approach for assessing bone strength in clinical studies? (2) What are the effects of exercise on biomarkers of osteocyte function? (3) Is the skeletal response to exercise attenuated or enhanced by use of medications (eg, nonsteroidal anti-inflammatory drugs [NSAIDs] or nitroglycerin) that act on signaling factors in mechanotransduction? (4) Do metabolic responses to exercise diminish the potential skeletal benefits by stimulating bone resorption, and can this effect be attenuated? and (5) What strategies or therapeutics are effective in increasing the local and/or generalized anabolic responses to exercise?

Mechanisms of Cellular Aging

The aging cell

Aging is the largest single risk factor for developing a full array of diseases in several organs and tissues, including bones and joints. Most age-related diseases, including the bone disease that develops with aging, are degenerative in nature; that is, they are associated with loss of tissue structure and function. An exception to this trend is age-related malignancies, hyperproliferative diseases that present with gain-of-function cellular and tissue phenotypes. Whether common or diverse mechanisms are involved in degenerative versus proliferative aging-related diseases is unknown. However, understanding the biology of both types of diseases may prove fundamental to postponing or treating multiple age-related pathologies.[50] A feature of aging and most age-related diseases is the accumulation of senescent cells, which exhibit paracrine actions that disrupt the structure and function of normal tissues.[51] Senescent cells acquire a distinct secretory phenotype (SASP) that, in turn, alters the tissue environment. The SASP is conserved among cell types and between humans and mice, validating the mouse as a model for studying the relationship between cellular senescence and aging. Cellular senescence arrests the proliferation of damaged cells or cells otherwise at risk for oncogenic transformation and thus is a tumor-suppressive response. However, the proinflammatory nature of the SASP can fuel tissue degeneration and, paradoxically, cancer progression.[52] Three major signaling pathways that regulate the SASP in human fibroblasts have been identified. They are the DNA damage response, the p38MAPK/NFkB axis, and the mTOR pathway. Inhibition of these pathways with pharmacologic as well as genetic tools partially reverses SASP. Thus, targeting these pathways may ameliorate the degenerative as well as proliferative diseases of aging. Important unresolved issues and directions for future work include (1) defining the possible mechanisms for (epi)genomic damage and the consequent inflammatory cytokine secretion in aging cells and (2) identifying the potential consequences of inhibiting local and systemic inflammatory responses to damage (SASP) in aging cells.

Autophagy and aging cartilage

Aging is a major risk factor for the development of osteoarthritis (OA), the most prevalent disease of the joints that affects, in particular, articular cartilage. Understanding the mechanisms by which joint homeostasis is regulated and the causes of aging-related joint disease may provide opportunities for OA prevention. Autophagy is a catabolic process by which cells degrade their own damaged and dysfunctional organelles and macromolecules through the lysosomal machinery. Absence of autophagy might trigger cellular apoptosis. In postmitotic tissues, such as cartilage, autophagy is a major mechanism for maintaining cell survival and normal tissue function. Autophagy is constitutively active and appears to play a protective role in maintaining articular cartilage.[53] Cells of the superficial zone of healthy, young articular cartilage express high levels of proteins that regulate autophagy, such as ULK1, beclin1, and LC3. In contrast, autophagy is decreased and apoptosis is increased in articular cartilage cells of the joints from humans with OA, old mice, or mice with surgically induced OA.[54] OA and cartilage injury induced by excessive mechanical stimulation are associated with reduced expression of ULK1, beclin1, and LC3 in the superficial zone. Activation of autophagy by inhibiting the mTOR pathway with rapamycin prevents cell death and the loss of extracellular matrix in murine models of OA. Thus pharmacologic interventions that enhance autophagy may protect articular cartilage after mechanical injury and inhibit aging-related cartilage cell death and dysfunction. Important unresolved issues and directions for future work include (1) identifying the overall mechanisms by which aging reduces autophagy and (2) defining the importance and role of autophagy and the mTOR pathway in mediating age-related changes in bone, cartilage, and muscle.

Regulation of life span and age-related diseases by dietary restriction

Dietary restriction (DR; 60% of *ad libitum*-fed mice) delays aging and extends life span, as well as health span, in all species tested. Among the mechanisms proposed for the effects of DR on life and health span is resistance to oxidative stress and attenuation of the onset of age-related diseases. Mice lacking the antioxidant enzyme CuZn superoxide dismutase (SOD) ($Sod1^{-/-}$ mice) have very high levels of oxidative stress and damage and show a significant reduction in lifespan, acceleration of age-related loss of skeletal muscle mass, and a high incidence of liver cancer.[55] DR increases $Sod1^{-/-}$ mice lifespan to levels comparable with wild-type mice fed *ad libitum*. The reduced death in $Sod1^{-/-}$ mice under DR results from decreases in both neoplastic and nonneoplastic diseases. In addition, the incidence of hepatocellular carcinoma is significantly lower in $Sod1^{-/-}$ mice under DR. DR also leads to reduced generation of reactive oxygen species by the mitochondria, maintenance of mitochondrial integrity, and lower levels of oxidative damage in muscles of DR $Sod1^{-/-}$ mice.[56] Moreover, the muscle atrophy observed in $Sod1^{-/-}$ mice fed *ad libitum* is attenuated by DR. $Sod1^{-/-}$ mice as well as aging mice under DR exhibit better-preserved neuromuscular junctions compared with their respective controls, suggesting that the reduction in sarcopenia observed in DR $Sod1^{-/-}$ mice may result from maintenance of neuromuscular junction integrity. In summary, DR is a powerful antiaging intervention that attenuates oxidative stress–induced age-related muscle loss, reduces pathology, and extends the lifespan of $Sod1^{-/-}$ mice. Future research will be important in (1) obtaining a better understanding the potential impact of DR on skeletal health, (2) defining the relative contribution of neuronal and muscular function to age-related sarcopenia, and (3) understanding whether muscle atrophy can be attenuated by regulating nerve conduction and myelination.

Muscle stem cell function in aging

Sarcopenia, the loss of skeletal muscle mass and strength, is associated with atrophy of myofibers, fibrosis, and intramuscular fat accumulation in aging. Reduced number and myogenic ability of satellite cells (muscle stem cells that reside under the myofiber basal lamina) is a hallmark of aging muscle and may be

a contributory factor to the age-associated decline in myofiber repair and decreased muscle mass.[57] Populations of satellite cells isolated from old rodents self-renew and produce myogenic progeny when cultured in rich medium or transplanted into young hosts, indicating maintenance of myogenic capacity by at least some of the aged cells and the importance of extrinsic stimuli in the age-associated decline in satellite cell function in vivo.[57–60] However, cell-autonomous intrinsic factors also appear to contribute to the decline in satellite cell performance with age, as evidenced by studies using single cells.[61] The decrease in satellite cells with age is found in males and females, but in both young and old mice and rats, females have fewer satellite cells than males.[57,61] Exercise enhances the number and myogenic performance of satellite cells and decreases the number of myofiber-associated nonmyogenic cells in aging muscle, suggesting that rejuvenating the aged niche influences satellite cell differentiation and performance.[57] Understanding the mechanisms by which the aging process modulates the properties of muscle stem cells is a prerequisite for developing new therapies for combating sarcopenia. Future research needs to focus on (1) understanding mechanisms to increase self-renewal and differentiation of muscle satellite cells toward mature muscle cells and (2) understanding mechanisms to reduce preadipogenic cells in aging muscle.

Understanding Physiologic Signals Contributing to Age-Related Bone Loss

Bone loss through the menopausal transition

There are varying definitions of *perimenopause*, and studies addressing bone loss through the menopausal transition would benefit from a more uniform definition. Studies using DXA with a longitudinal approach[62–64] have found little change in BMD in early menopausal women, but rates of bone loss accelerate in late perimenopause and appear to slow several years after the final menstrual period. However, many existing longitudinal studies are limited by small sample sizes, short follow-up, variations in the definition of menopause status, use of older bone density technologies, and/or changes in measurement techniques. The Study of Women's Health Across the Nation (SWAN) is a multicenter study examining a wide variety of issues in a diverse racial and ethnic cohort. Data from SWAN indicate that bone loss at the spine and hip increase from premenopause and early perimenopause to late perimenopause to postmenopause. This pattern is essentially similar across the racial groups studied (ie, whites, African Americans, Chinese, and Japanese), although the rates of loss do vary somewhat by racial group.[26] Data from SWAN[65] and other cohorts[64,66] indicate that (1) bone loss at menopause appears greatest at trabecular sites, (2) there may be compensatory increases in bone size, (3) the rate of bone loss is lower in obese women, (4) both serum follicle-stimulating hormone (FSH) and estradiol levels are correlated with rates of bone loss, and (5) menopause may be associated with changes in muscle strength. Critical research gaps and questions identified include: (1) What measures can identify midlife women at highest risk of fracture and faster rates of bone loss through menopause in order to target treatment? (2) What are the mechanisms for the ethnic differences in the rates of bone loss? and (3) What additional data are needed using state-of-the-art methods to define changes in bone structure (geometry, size, and strength) through the menopausal transition?

Oxidative stress and age-related bone loss

A major imperative for bone research in aging is whether skeletal involution is an inexorable accompaniment of longevity or can be altered by targeting molecular pathways and mechanisms of aging so that "bone health span" can increase in tandem with lifespan. Studies in mice have shown that advancing age increases markers of oxidative stress in bone.[67] Products of oxidative stress, including reactive oxygen species (ROS), attenuate osteoblastogenesis and decrease osteoblast/osteocyte lifespan; conversely, ROS are required for osteoclast generation, function, and survival. Oxidative stress inhibits bone formation, in part, by antagonizing Wnt signaling by diverting β-catenin from TCF- to FoxO-mediated transcription.[68] At least in mice, the effects of aging on oxidative stress are recapitulated by the loss of sex steroids, and interestingly, the effects of sex steroid deficiency on bone are reversed by antioxidants.[67] Age-related activation of PPARγ by ligands generated from free fatty acid oxidation also leads to an attenuation of Wnt signaling and a decrease in bone formation.[69] In addition, increased glucocorticoid production and sensitivity with advancing age decrease skeletal hydration and thereby increase skeletal fragility secondary to attenuating vascular endothelial growth factor production by osteoblasts/osteocytes, the volume of the bone vasculature, and skeletal fluid flow.[70] Finally, autophagy in aging osteocytes may play a critical role in the maintenance of bone mass. Collectively, these findings highlight intrinsic, cell-autonomous changes in bone that interact with the effects of sex steroid deficiency to culminate in bone loss. Critical questions identified for future research include: (1) What are the molecular mechanisms of the adverse effects of aging on bone, and can these be attenuated by antioxidants? (2) How do estrogen deficiency and aging influence each other's negative impact on bone? and (3) Are there drugs targeting pathways to simultaneously prevent osteoporosis and other degenerative diseases?

Role of T cells and other immune cells in estrogen-deficiency bone loss

T cells are known to secrete osteoclastogenic cytokines and have been implicated in the bone loss induced by infection and inflammation. However, T cells also express estrogen receptors and estrogen deprivation leads to T-cell activation.[71,72] The most direct evidence for a role of T cells in mediating estrogen-deficiency bone loss in mice comes from studies showing that ovariectomy does not induce bone loss in mice depleted of T cells with anti-T-cell antibodies.[73] In addition, mice treated with CTLA4-Ig,[74] an immunosuppressant that causes T-cell anergy and apoptosis, as well as mice deficient in CD40L,[73] a surface molecule of T cells required for T-cell activation, are protected against ovariectomy-induced bone loss. Activation of T cells by ovariectomy increases T-cell production of tumor necrosis factor

(TNF).[71,72] a cytokine that stimulates osteoclast formation by potentiating the activity of RANKL and by promoting the production of RANKL by osteoblastic cells. In addition, activation of CD40 signaling induced by T-cell-expressed CD40L expands bone marrow stromal cells, promotes osteoblast differentiation, and regulates osteoblast production of macrophage colony–stimulating factor (M-CSF), RANKL, and osteoprotegerin (OPG). Following ovariectomy, activated T cells home preferentially near endosteal surfaces to support the nearby formation of osteoclasts. Recent studies also have shown that ovariectomy leads to an accumulation of ROS, which then leads to an expansion of T cells owing to increased presentation to T cells of antigen fragments bound to major histocompatability (MHC) molecules expressed on antigen-presenting cells (ie, macrophages and dendritic cells). Key research questions identified include: (1) Do T cells play a significant causal role in postmenopausal osteoporosis or in other forms of bone loss (eg, primary hyperparathyroidism, steroid osteoporosis) in humans? (2) Does estrogen deficiency cause a nonspecific increase in T-cell reactivity to antigenic peptides, or does it cause the generation of new antigens? (3) What drives the homing of T cells to endosteal bone surfaces, and do T cells localize preferentially to areas in need of remodeling? and (4) Can inhibitors of costimulation be used to prevent postmenopausal bone loss?

Role of the CNS in mediating age-related bone loss

The CNS coordinately regulates bone mass by neuroendocrine and neuronal mechanisms. Thus the hypothalamic-pituitary axes regulate sex steroids, IGF-1, and cortisol production, each of which modulates bone metabolism. In addition, the sympathetic nervous system (SNS) has potent catabolic effects on bone and is, in turn, modulated in the hypothalamus by neuropeptides. Leptin, an adipocyte-derived satiety hormone, reduces bone mass via a central SNS circuit, stimulating the osteoblastic β_2-adrenergic receptor to activate the molecular clock and control timing of osteoblast proliferation.[75] This central effect of leptin is due to inhibition of serotonergic neurons.[76] Other hypothalamic neuropeptides involved in SNS regulation of bone mass include the cocaine- and amphetamine-regulated transcript,[77] the cannabinoid type I (CBI) receptor,[78] and neuropeptide Y (NPY).[79] Interactions between NPY and SNS circuits, between NPY and sex steroids, and between CNS modulation and more focal stimuli such as mechanical loading underscore the complexity of integrated physiologic responses underlying CNS regulation of bone mass. However, despite the many indications that neuronal pathways regulate bone homeostasis, the evidence for CNS involvement in age-related bone loss is inconclusive. As such, key areas for future research include (1) obtaining better clinical evidence for CNS control of bone in humans, (2) identifying age-related changes in CNS function that are contributors to age-related bone loss, (3) determining the best ways to therapeutically target the CNS-related bone regulatory circuits to achieve increased bone mass, and (4) clarifying CNS circuit interactions with endocrine/paracrine regulators of bone mass and exploring neural pathways for potential targets for combination therapies.

Disclosures

The American Society for Bone and Mineral Research (ASBMR) is well served by the fact that many of those responsible for policy development and implementation have diverse interests and are involved in a variety of activities outside the society. The ASBMR protects itself and its reputation by ensuring impartial decision making. Accordingly, the ASBMR requires that all ASBMR officers, councilors, committee chairs, editors-in-chief, associate editors, and certain other appointed representatives disclose any real or apparent conflicts of interest (including investments or positions in companies involved in the bone and mineral metabolism field), as well as any duality of interests (including affiliations, organizational interests, and/or positions held in entities relevant to the bone and mineral metabolism field and/or the American Society for Bone and Mineral Research).

Acknowledgments

The task force would like to thank the staff of the ASBMR, in particular Ann Elderkin, Gretchen Bretsch, and Stacey Barnes, for help with all aspects of this effort.

Authors' roles: Each of the listed authors participated in the conception and design of the meeting, participated in drafting the manuscript and revising it critically for important intellectual content, and approved the final version of the submitted manuscript.

The workshop was supported, in part, by Grant U13AG037272 from the National Institute on Aging, the National Institute of Child Health and Human Development, and the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Appendix: Participants in the Workshop

Bone accretion and loss: Catherine M Gordon, Children's Hospital Boston and Harvard Medical School, Boston, MA; Karen K Winer, National Institute of Child Health and Human Development, NIH, Bethesda, MD; Frank Rauch, Shriners Hospital for Children, Montreal, Canada; Heather A McKay, University of British Columbia, Vancouver, Canada; Mary B Leonard, Children's Hospital of Philadelphia, Philadelphia, PA; Marie Demay, Massachusetts General Hospital, Boston, MA; Arline Bohannon, Virginia Commonwealth University, Richmond, VA; Lynda F Bonewald, University of Missouri, Kansas City, MO. *Genetic and other risk factors for bone loss and fracture:* Douglas P Kiel, Institute for Aging Research, Hebrew Senior Life, Boston, MA; Sherry S Sherman, National Institute on Aging, NIH, Bethesda, MD; Andre G Uitterlindenm, Erasmus University, Rotterdam, The Netherlands; Anne Looker, National Center for Health Statistics, Hyattsville, ND; Keith A Hruska, Washington University at St Louis, St Louis, MO; Paul T Lips, VU University Medical Center, Amsterdam, The Netherlands; Laurence Z Rubenstein, Oklahoma University Health Science Center, Oklahoma City, OK; Robert Pignolo, University of Pennsylvania, Philadelphia, PA; Charlotte A Peterson, University of Kentucky, Lexington, KY. *Treatment approaches for aging and bone:* Clifford J Rosen, Maine Medical Center Research Institute, Scarborough, ME; Jay S Magaziner, University

Name	Affiliation	Conflicts	Commercial entity/ no. of relationships
Sundeep Khosla	College of Medicine, Mayo Clinic	Yes	Bone Therapeutics 2; Amgen 2; Pfizer 2
Teresita M Bellido	Indiana University School of Medicine	Yes	Amgen 1
Marc K Drezner	University of Wisconsin–Madison	No	None
Catherine M Gordon	Children's Hospital Boston, Harvard Medical School	Yes	Pfizer 6, Merck 6 (Co-Director, Clinical Investigator Training Program, Harvard Medical School with Pfizer/Merck)
Tamara B Harris	Intramural Research Program, Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging	No	None
Douglas P Kiel	Institute for Aging Research, Hebrew SeniorLife and Harvard Medical School	Yes	Amgen 1,2; Lilly 2; Merck 1,2; Novartis 2,
Barbara E Kream	University of Connecticut Health Center, Farmington, CT	Yes	Editorial Board, *Bone* 6
Meryl S LeBoff	Brigham and Women's Hospital, Harvard Medical School	Yes	Eli Lilly 2; Amgen 5, GE 5
Jane B Lian	University of Massachusetts Medical School, Department of Cell Biology	No	None
Charlotte A Peterson	College of Health Sciences, University of Kentucky	No	None
Clifford J Rosen	Maine Medical Center Research Institute	No	None
John P Williams	National Institute on Aging, NIH	No	None
Karen K Winer	Eunice Kennedy Shriver National Institute of Child Health and Human Development	No	None
Sherry S Sherman	National Institute on Aging, NIH	No	None

Relationship key:
1 = Research grant or financial support from commercial entities.
2 = Consultant or member of advisory board to a commercial entity.
3 = Participant in a speaker's bureau.
4 = Employment or executive positions in pharmaceutical, medical device, or diagnostic companies.
5 = Stock holdings in pharmaceutical, medical device, or diagnostic companies.
6 = Any other situation or transaction in which you have a formal role or interest (eg, you serve on a bone-related organization's board, committee, or journal; a family member contracts with ASBMR, etc.).

of Maryland, Baltimore, MD; Richard A Miller, University of Michigan, Ann Arbor, MI; Susan L Greenspan, University of Pittsburgh, Pittsburgh, PA. *Aging-related changes in bone structure and cellular activity:* Meryl S LeBoff, Brigham and Women's Hospital, Boston, MA; Orhan K Oz, University of Texas Southwestern Medical Center, Dallas, TX; Nicola Crabtree, Queen Elizabeth Hospital, Birmingham, UK; Mary L Bouxsein, Beth Israel Deaconess Medical Center, Boston, MA; Lynda F Bonewald, University of Missouri, Kansas City, MO; Wendy M Kohrt, University of Colorado, Denver, CO; Elizabeth Shane, Columbia University, New York, NY; Roberto Pacifici, Emory University School of Medicine, Atlanta, GA. *Mechanisms of cellular aging:* Teresita M Bellido, Indiana University, Indianapolis, IN; John P Williams, National Institute on Aging, NIH, Bethesda, MD; Judith Campisi, Lawrence Berkeley Laboratory, Berkeley, CA; Martin Lotz, Scripps Research Institute, La Jolla, CA; Holly Van Remmen, University of Texas Health Science Center, San Antonio, TX; Zipora Yablonka-Reuveni, University of Washington School of Medicine, Seattle, WA; James Kirkland, Mayo Clinic, Rochester, MN; Tamara B Harris, National Institute on Aging, Bethsda, MD. *Understanding physiologic signals contributing to age-related bone loss:* Sundeep Khosla, Mayo Clinic, Rochester, MN; Marja M Hurley, University of Connecticut, Farmington, CT; Jane A Cauley, University of Pittsburgh, Pittsburgh, PA; Stavros C Manolagas, University of Arkansas, Little Rock, AK; Roberto Pacifici, Emory University School of Medicine, Atlanta, GA; Edith M Gardiner, University of Washington, Seattle, WA; Thomas L Clemens, John Hopkins University, Baltimore, MD; Jane B Lian, University of Massachusetts Medical School, Worcester, MA.

References

1. US Department of Health and Human Services. The frequency of bone disease. In: *Bone health and osteoporosis: a report of the surgeon general*. Rockville, MD: US Department of Health and Human Services, Office of the Surgeon General; 2004, pp 68–87.
2. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9:1137–41.
3. National Osteoporosis Foundation (NOF). *America's bone health: the state of osteoporosis and low bone mass in our nation*. Washington, DC: NOF; 2002.
4. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res. 2007;22:465–75.
5. Rauch F, Neu C, Manz F, Schoenau E. The development of metaphyseal cortex: implications for distal radius fractures during growth. J Bone Miner Res. 2001;16:1547–55.
6. Wang Q, Nicholson PHF, Suuriniemi M, Lyytikainen A, Helkala E, Alen M, Suominen H, Cheng S. Relationship of sex hormones to bone geometric properties and mineral density in early pubertal girls. J Clin Endocrinol Metab. 2004;89:1698–703.

7. Rauch F, Schoenau E. Changes in bone density during childhood and adolescence: an approach based on bone's biologic organization. J Bone Miner Res. 2001;16:597–604.
8. McKay HA, Smith E. Winning the battle against childhood physical inactivity: the key to bone strength? J Bone Miner Res. 2008;23:980–5.
9. Nikander R, Sievanen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted excerside against osteoporosis: a systemic review and meta-analysis for optimising bone strength throughout life. BMC Med. 2010;8:47.
10. Lewiecki EM, Gordon CM, Baim S, Leonard MB, Bishop NJ, Bianchi M-L, Kalkwarf H, Langman CB, Plotkin H, Rauch F, Zemel BS, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Silverman S. International Society for Clinical Desitometry 2007 adult and pediatric official postions. Bone. 2008;43:1115–21.
11. Dubner SE, Shults J, Baldassano RN, Zemel B, Thayu M, Burnham JM, Herskovitz RM, Howard KM, Leonard MB. Longitudinal assessment of bone density and structure in an insident cohort of children with Crohn's disease. Gastroenterology. 2009;136:123–30.
12. Thayu M, Leonard MB, Hyams J, Crandall WV, Kugathasan S, Otley AR, Olson A, Johanns J, Marano CW, Heuschkel RB, Veereman-Wauters G, Griffiths AM, Baldassano RN, Group RS. Improvement in biomarkers of bone formation during infliximab therapy in pediatric Crohn's disease: results of the REACH study. Clin Gastroenterol Hepatol. 2008;6:1378–84.
13. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, Mahboubi S, Shepard JA, Hangartner TN, Frederick MM, Winer KK, Kalkwarf HJ. Height adjustment in assessing dual energy X-ray absorptiometry measurements of bone mass and density in children. J Clin Endocrinol Metab. 2010;95:1265–73.
14. Demay MB, Sabbagh Y, Carpenter TO. Calcium and vitamin D: what is known about effect on growing bone. Pediatrics. 2007;119(Suppl 2): S141–4.
15. Miedlich SU, Zhu ED, Sabbagh Y, Demay MB. The receptor-dependent actions of 1,25-dihyroxyvitamin D are required for normal growth plate maturation in *Npt2a* knockout mice. Endocrinology. 2010;151:4607–12.
16. Ecklund K, Vajapeyam S, Feldman HA, Buzney CD, Mulkern RV, Kleinman PK, Rosen CJ, Gordon CM. Bone marrow changes in adolescent girls with anorexia nervosa. J Bone Miner Res. 2010;25: 298–304.
17. Gordon CM, Gordon LB, Snyder BD, Nazarian A, Quinn N, Huh S, Giobbie-Hurder A, Neuberg D, Cleveland R, Kleinman M, Miller DT, Kieran MW. Hutchinson-Gilford progeria is a sleletal dysplasia. J Bone Miner Res. 2011;26:1670–1679.
18. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, WIlson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG, Consortium GFfOG. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009;41: 1199–206.
19. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quatitative traits. Nat Genet. 2009;41:527–34.
20. Hsu YH, Zillikens MC, WIlson SG, Farber CR, Demissie S, Soranzo N, Bianchi EN, Grundberg E, Liang L, Richards JB, Estrada K, Zhou Y, van Nas A, Moffatt MF, Zhai G, Hofman A, van Meurs JB, Pols HAP, Price RI, Nilsson O, Pastinen T, Cupples LA, Lusis AJ, Schadt EE, Ferrari S, Uitterlinden AG, Rivadeneira F, Spector TD, Karasik D, Kiel DDP. An integration of genome-wide assosication study and gene expression profiling to prioritize the discover of novel susceptibility Loct for osteoporosis-related traits. PLoS Genet. 2010;6:e1000977.
21. Baron JA, Karagas M, Barrett J, Kniffin W, Malenka D, Mayor M, Keller RB. Basic epidemiology of fractures of the upper and lower limb among Americans over 65 years of age. Epidemiology. 1996;7:612–8.
22. Lauderdale DS, Jacobsen SJ, Furner SE, Levy PS, Brody JA, Goldberg J. Hip fracture incidence among elderly Hispanics. Am J Public Health. 1998;88:1245–7.
23. Lauderdale DS, Jacobsen SJ, Furner SE, Levy PS, Brody JA, Goldberg J. Hip fracture incidence among elderly Asian-American populations. Am J Epidemiol. 1997;146:502–9.
24. Cauley JA, Liu LY, Stone KL, Hillier TA, Zmuda JM, Hochberg M, Beck TJ, Ensrud KE. Longitudinal study of changes in hip bone mineral density in Caucasian and African-American women. J Am Geriatr Soc. 2005;53:183–9.
25. Ross PD, He YF, Yates AJ, Coupland C, Ravn P, McClung M, Thompson D, Wasnich RD. Body size accounts for most differences in bone density between Asian and Caucasian women. Calcif Tissue Int. 1996;59:339–43.
26. Finkelstein JS, Brockwell SE, Mehta V, Greendale GA, Sowers MR, Ettinger B, Lo JC, Johnston JM, Cauley JA, Danielson ME, Neer RM. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab. 2008;93: 861–68.
27. Pereira RC, Juppner H, Azucena-Serrano CE, Yadin O, Salusky IB, Wesseling-Perry K. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. Bone. 2009;45:1161–8.
28. Oliveira RB, Cancela AL, Graciolli FG, Dos Reis LM, Draibe SA, Cuppari L, Carvalho AB, Jorgetti V, Canziani ME, Moyses RM. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? Clin J Am Soc Nephrol. 2010;5:286–91.
29. LaCroix AZ, Lee JS, Wu L, Cauley JA, Shlipak MG, Ott SM, Robbins J, Curb JD, Leboff M, Bauer DC, Jackson RD, Kooperberg CL, Cummings SR, Observational WsHI. Cyctatin-C, renal function, and incidence of hip fracture in postmenopausal women. J Am Geriatr Soc. 2008;56: 1434–41.
30. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J. Fall prevention with supplement and active forms of vitamine D: a meta-analysis of randomised controlled trials. BMJ. 2009;339:b3692.
31. Gillespie LD, Robertson MC, Gillespie WJ, Lamb SE, Gates S, Cumming RH, Rowe BH. Interventions for preventing falls in older people living in the community. Cochrane Database Syst Rev. 2009;2:CD007146.
32. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC. Annual high-dose oral vitamine D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010;303:1815–22.
33. Lips P, Binkley N, Pfeifer M, Recker R, Samanta S, Cohn DA, Chandler J, Rosenberg E, Papanicolaou DA. Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D unsufficiency. Am J Clin Nutr. 2010;91:985–91.
34. Rubenstein LZ, Josephson KR. The epidemiology of falls and syncope. Clin Geriatr Med. 2002;18:141–58.
35. Stevens JA, Baldwin GT. An older adult falls reserach agenda from a public health perspective. Clin Geriatr Med. 2010;26:767–79.
36. Michael YL, Whitlock EP, Lin JS, Fu R, O'Connor EA, Gold R. Primary care-relevant interventions to prevent falling in older adults: a systematic evidence review for the US Preventive Services Task Force. Ann Intern Med. 2010;153:815–25.
37. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA,

Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature. 2009;460:392–5.

38. Frost HM. Bone "mass" and the "mechanostat": A proposal. Anat Rec. 1987; Jan; 219:9.
39. Leonard MB, Elmi A, Mostoufi-Moab S, Shults J, Burnham JM, Thayu M, Kibe L, Wetzsteon RJ, Zemel BS. Effects of sex, race, and puberty on cortical bone and the functional muscle bone unite in children, adolescents, and young adults. J Clin Endocrinol Metab. 2010;95: 1681–9.
40. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. J Bone Miner Res. 2008;23:1946–53.
41. Bouxsein ML, Melton LJ III, Riggs BL, Muller J, Atkinson EJ, Oberg AL, Robb RA, Camp JJ, Rouleau PA, McCollough CH, Khosla S. Age- and sex-specific differences in the factor of risk for vertebral fracture: a population-based study using QCT. J Bone Miner Res. 2006;21: 1475–82.
42. Keaveny TM, Kopperdahl DL, Melton LJ III, Hoffmann PF, Amin S, Riggs BL, Khosla S. Age-dependence of femoral strength in white women and men. J Bone Miner Res. 2010;25:994–1001.
43. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab. 2007;5: 464–75.
44. Bonewald LF. Osteocyte messages from a bony tomb. Cell Metab. 2007;5:410–1.
45. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26: 229–38.
46. Bonaiuti D, Shea B, Iovine R, Negrini S, Robinson V, Kemper HC, Wells G, Tugwell P, Cranney A. Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev. 2002; (2):CD000333.
47. Kohrt WM, Ehsani AA, Birge SJJ. Effects of exercise involving predominantly either joint-reaction or ground-reaction forces on bone mineral density in older women. J Bone Miner Res. 1997;12:1253–61.
48. Barry DW, Kohrt WM. BMD decreases over the course of a year in competitive male cyclists. J Bone Miner Res. 2008;23:484–91.
49. Barry DW, Hansen KC, van Pelt RE, Witten M, Wolfe P, Kohrt WM. Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. Med Sci Sports Exerc. 2011;43:617–23.
50. Vijg J, Campisi J. Puzzles, promises and a cure for ageing. Nature. 2008;454:1065–71.
51. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med. 2010;16:238–46.
52. Rodier F, Campisi J. Four faces of cellular senescence. J Cell Biol. 2011;192:547–56.
53. Carames B, Taniguchi N, Otsuki S, Blanco FJ, Lotz M. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. Arthritis Rheum. 2010; 62:791–801.
54. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilabe degradation in human osteoarthritis. Arthritis Rheum. 1998;41:1632–8.
55. Lustgarten MS, Jang YC, Liu Y, Qi W, Qin Y, Dahia PL, Shi Y, Bhattacharya A, Muller FL, Shimizu T, Shirasawa T, Richardson A, Van Remmen H. MnSOD deficiency results in elevated oxidative stress and decreased mitochondrial function but does not lead to muscle atrophy during aging. Aging Cell. 2011;10:493–505.
56. Sakellariou GK, Pye D, Vasilaki A, Zibrik L, Palomero J, Kabayo T, McArdle F, Van Remmen H, Richardson A, Tidball JG, McArdle A, Jackson MJ. Role of superoxide-nitric oxide interactions in the accelerated age-related loss of muscle mass in mice lacking Cu, Zn superoxide dismutase. Aging Cell. DOI: 10.1111/j.1474-9726.2011.00709.x.
57. Shefer G, Rauner G, Yablonka-Reuveni Z, Benayahu D. Reduced satellite cell numbers and mygenic capacity in aging can be alleviated by endurance excersise. Plos ONE. 2010;5:e13307.
58. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005;433:760–4.
59. Collins CA, Zammit PS, Ruiz AP, Morgan JE, Partridge TA. A population of myogenic stem cells that survives skeletal muscle aging. Stem Cells. 2007;25:885–94.
60. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007;317:807–10.
61. Day K, Shefer G, Shearer A, Yablonka-Reuveni Z. The depletion of skeletal muscle satellite cells with ate is concomitant with reduced capacity of single progenitors to produce reserve progeny. Dev Biol. 2010;340:330–43.
62. Slemenda C, Hui SL, Longcope C, Johnston CC. Sex steroids and bone mass: a study of changes about the time of menopause. J Clin Invest. 1987;80:1261–9.
63. Recker R, Lappe J, Davies K, Heaney R. Characterization of perimenopausal bone loss: a prospective study. J Bone Miner Res. 2000;15: 1965–73.
64. Guthrie JR, Dennerstein L, Taffe JR, Lehert P, Burger HG. The menopausal transition: a 9-year prospective population-based study. The Melbourne Women's Midlife Health Project. Climacteric. 2004;7: 375–89.
65. Sowers MFR, Jannausch M, McConnell D, Little RD, Greendale GA, Finkelstein JS, Neer RM, Johnston J, Ettinger B. Hormone predictors of bone mineral density changes during the menopausal transition. J Clin Endocrinal Metab. 2006;91:1261–7.
66. Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone loss and bone size after menopause. N Engl J Med. 2003;349: 327–34.
67. Almeida M, Martin-Millan M, Plotkin LI, Stewart SA, Roberson PK, Kousteni S, O'Brien CA, Bellido T, Parfitt AM, Weinstein RS, Jilka RL, Manolagas SC. Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. J Biol Chem. 2007;282: 27285–97.
68. Manolagas SC, Almeida M. Gone with the Wnts: β-catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism. Mol Endocrinol. 2007;21:2605–14.
69. Almeida M, Ambrogini E, Han L, Manolagas SC, Jilka RL. Increased lipid oxidation causes oxidative stress, increased peroxisome proliferator-activated receptor-γ expression, and diminished pro-osteogenic Wnt signaling in the skeleton. J Biol Chem. 2009;284:27438–48.
70. Weinstein RS, Wan C, Liu Q, Wang Y, Almeida M, O'Brien CA, Thostenson J, Roberson PK, Boskey AL, Clemens TL, Manolagas SC. Endogenous glucocorticoids decrease skeletal angiogenesis, vascularity, hydration, and strength in aged mice. Aging Cell. 2010;9: 147–61.
71. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-α. J Clin Invest. 2000;106:1229–37.
72. Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R. Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci USA. 2001;98:13960–5.
73. Li J-Y, Tawfeek H, Bedi B, Yang X, Adams J, Gao KY, Zayzafoon M, Weitzmann MN, Pacifici R. Ovariectomy disregulates osteoblast and osteoclast formation through the T-cell receptor CD40 ligand. Proc Natl Acad Sci USA. 2011;108:768–73.

74. Grassi F, Tell G, Robbie-Ryan M, Gao Y, Terauchi M, Yang X, Romanello M, Jones DP, Weitzmann MN, Pacifici R. Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation. Proc Natl Acad Sci USA. 2007;104:15087–92.
75. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. Cell. 2005;122:803–15.
76. Yadav VK, Oury F, Suda N, Liu Z-W, Gao X-B, Confavreux C, Klemenhagen KC, Tanaka KF, Gingrich JA, Guo XE, Tecott LH, Mann JJ, Hen R, Horvath TL, Karsenty G. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. Cell. 2009;138:976–89.
77. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature. 2005;434:514–20.
78. Ofek O, Karsak M, Leclerc N, Fogel M, Frenkel B, Wright K, Tam J, Attar-Namdar M, Kram V, Shohami E, Mechoulam R, Zimmer A, Bab I. Peripheral cannabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci USA. 2006;103:696–701.
79. Baldock PA, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, Herzog H. Hypothalmamic Y2 receptors regulate bone formation. J Clin Invest. 2002;109:915–21.