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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 Rosen, John. P. Williams, Karen K. Winer, and Sherry S. Sherman

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 adequately evaluated 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 this meeting and in each area, identifies key questions to help set the agenda for future research in skeletal aging.

Keywords

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: 10 million Americans 50 yrs old already have osteoporosis by World Health Organization (WHO) criteria (2), while 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 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 have not, for the most part, been adequately applied 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 on 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)

Corresponding authors: Sundeep Khosla, M.D., Mayo Clinic, Endocrine Research Unit and Kogod Center on Aging, 200 First Street SW, Guggenheim 7, Rochester, MN 55905. Phone: (507) 255-6663, Fax: (507) 293-3853, khosla.sundeep@mayo.edu; Sherry S. Sherman, Ph.D., National Institute on Aging, Gateway Building, Suite 3C-307, 7201 Wisconsin Avenue MSC 9205, Bethesda, MD 20892. Phone: (301) 496-6942, Fax: (301) 402-1784, shermans@nia.nih.gov.

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Name	Affiliation	Conflicts	Commercial Entity/# 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 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

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.

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 due to the interplay of genetic, hormonal, nutritional and mechanical factors. The skeleton undergoes marked changes in both length and width, which 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 due 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 cyclical 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 due 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 biological 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 to 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 have also not controlled for maturity, a critically important factor to consider as 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 is 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 due to chronic illness. As noted earlier, DXA is a two-dimensional projection technique that estimates areal bone mineral density (BMD) and underestimates volumetric BMD 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 (glucocorticoids) are associated with bone and muscle deficits. pQCT provides measures of trabecular and cortical volumetric bone mineral density, as well as valuable information on cortical dimensions (11). Deficits in trabecular volumetric BMD, cortical bone geometry and muscle have been observed in Crohn's disease at the time of diagnosis. Tumor necrosis factor-alpha (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, and increases in height Z-scores (12).

Chronic illness may lead to delayed linear growth and sexual maturation which result in inaccurate DXA measures due to a systematic under-estimation of volumetric density in children who are small. Zemel et al (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 non-invasive measures of bone health (e.g., DXA, pQCT, 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-term 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 (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 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 caspase-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 production (15). However, VDR/Npt2a double knockout mice exhibit severe rickets. Therefore, receptor-dependent actions of 1,25(OH)₂D 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 analogs 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 on a growing and adult skeleton?

Effect of nutritional deprivation on bone

The childhood and adolescent years are critical ones for the achievement of peak bone mass. Growth, pubertal development and bone accrual should occur simultaneously in a healthy adolescent and are nutrition-dependent physiological 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 I 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 between 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 most recently focused on genome-wide association studies. 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-analysis 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-analysis of candidate gene association studies have shown that variants in the *ESR1*, the *Col1A1*, *VDR*, and *LRP5* genes are associated with either fractures in general or with vertebral fractures. The study of fracture phenotypes using GWAS has been less well developed; however, 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 were used to determine if these SNPs were also associated with fractures. In 14 out of the 96 SNPs that were tested there was evidence of association with fractures with odds ratios 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: 1) Taking the findings from GWAS to understand what the causal SNPs and genes really are and understanding the biologic mechanism underlying the findings; 2) Since the current findings have explained a limited percent of the variance in the 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 includes 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 of genetic loci for bone phenotypes other than BMD and/or fracture; 4) Role of pleiotropic genetic effects; 5) Deeper analysis of heterogeneity in GWAS signals; 6) Role of genetics in the determination of peak bone mass and rates of bone loss (peri-menopausal; age-related) for future fracture risk; 7) Genetics of response-to-treatment; and 8) 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 amongst different race/ethnic groups. Differences between race/ethnic groups can result from biological, behavioral, and cultural sources. There is also evidence of genetic admixture in all four major race/ethnic groups in the US. As a result, identifying the reason for variability between race/ethnic groups can be complicated. Currently 81% of those 65 years and older are non-Hispanic whites (NHW), but this proportion is expected to decline to 60% by the year 2050, as significant increases in nonwhite groups - especially amongst Hispanic populations - are expected in the US population. Most fracture-related data from non-whites focuses on blacks where hip fractures, clinical vertebral fractures and fractures 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 whites; limited data suggest this may also 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 than whites, whereas Asians have lower fracture risk despite lower BMD 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 to whites depending on skeletal site and sex (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 which can explain the observed differences in fracture risk between 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, in body composition, in bone geometry, and in fall risk.

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

About 30% of patients over the age of 65 years with decreased BMD also have decreased estimated glomerular filtration rate (eGFR), and about 26 million Americans have decreased eGFR (stage 2-3 Chronic Kidney Disease [CKD]), most with no history of actual kidney disease. Individuals with decreased BMD and eGFR may either have underlying kidney disease or may simply have 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 PTH. Also, early on, FGF23 and sclerostin levels are increased, which results in decreases in 1,25 dihydroxyvitamin D production and a decrease in bone formation rate. The early onset of the CKD-MBD is reinforced by development of the classical pathophysiology of secondary hyperparathyroidism as the kidney disease progresses, leading to the 17-fold increase in hip fracture risk in the CKD-MBD (27,28).

In contrast to the CKD-MBD pathogenesis, 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; however, this is an area that needs more research. Areas identified for further research include: 1) Determining if elderly patients with decreased BMD and eGFR have kidney disease; 2) Identifying direct effects of the aging kidney on the skeleton; 3) Determining whether patients with the aging kidney have the CKD-MBD (specifically, are Wnt inhibitors elevated? Is FGF23 increased? Is sclerostin increased? Are leptin, serotonin, etc. affected?); 4) Defining effects of skeletal anabolic therapy on cardiovascular risk in elderly patients with decreased BMD and eGFR; 5) Establishing consensus on the pathogenesis of the CKD-MBD; 6) Validation of new biomarkers in the CKD-MBD and clinical implementation of their use (FGF23, vascular calcium, DKK1, etc.); and 7) New therapeutic approaches to the CKD-MBD (all of the approved pharmacologic agents are labelled 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 six months, suggesting that the intervention may have reduced falls. In observational studies addressing the relation between serum 25 hydroxy-vitamin 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 of 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) might actually 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 of 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) Are falls associated with polymorphisms of vitamin D-related genes?; and 4) Defining whether vitamin D prevent 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 multi-factorial fall risk assessments, targeted exercise and mitigation of physiologic deficiencies (e.g. vitamin D) and environmental hazards (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 identified 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 which results in fractures; 2) Evaluating causation versus association and modifiability of risk factors in the home and institutional setting and 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 are generally 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 bone mineral density, few studies have determined who in these cohorts are at highest risk and thus who should be treated with anti-osteoporosis drugs. Even more remarkable is the lack of randomized clinical trials for osteoporosis interventions in LTCs. There are several reasons for the paucity of trials in these cohorts, including the many co-morbidities 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 of a university setting. Thus the pertinent clinical questions and research gaps are: 1) Identifying who are at the greatest risk for fracture in LTC?; 2) What is the most cost-effective means of conducting osteoporosis trials in the frail elderly?; 3) How can randomized, placebo-controlled clinical trials be practically performed in LTC facilities?; and 4) What are 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 nine 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-I, 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-I conditions) are associated with greater longevity in mice. In contrast, by mid-life 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, 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 1 to IV; however, 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 (e.g., 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 the 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 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 was greater in women than 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 due 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 (e.g., 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 may also 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 impact hip fracture risk include fall force, soft tissue thickness and muscle strength. Vertebral loading that may impact 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 to 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 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 due 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 >90-95% of all bone cells in the adult skeleton. They survive for decades and have long dendritic processes and complex lacuno-canalicular 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 the Wnt/ β -catenin pathway. Research studies indicate that osteocytes participate in the following functions: 1) Control of mineralization [promote mineralization and bone formation (with expression of *Phex* and *Dmp1*) or inhibit mineralization and bone formation (with expression of *Sost/sclerostin* and *MEPE/OF45*); 2) Regulate phosphate homeostasis and secretion of FGF-23; 3) Contribute to calcium homeostasis; 4) Regulate osteoblast activity (*Sost/sclerostin* are late osteocyte-selective factors); 5) Recruit osteoclasts in which osteocyte viability and cell death plays a role; and 6) Contribute to muscle myogenesis potentially through the production of secreted factors that affect muscle cells (e.g., C2C12 cells) [reviewed in (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 may also regulate muscle cells through secreted factors, changes in osteocyte function may also 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 1-3% 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: 9-14.9 MET-h/wk of physical activity; 1290 kcal/wk; or 3-4 hours of walking/wk. Although recommendations for exercise generally include weight bearing endurance activities 3 to 5 times/wk and resistance exercises 2 to 3 times/wk, 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 because they participate in weight-supported (e.g., cycling or swimming), rather than weight-bearing (e.g., running or gymnastics) activities. However, BMD 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 parathyroid hormone 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 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 (e.g., 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 might prove fundamental to postpone or treat 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

pro-inflammatory 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 pharmacological as well as genetic tools partially reverses SASP. Thus, targeting these pathways might 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 the 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, pharmacological interventions that enhance autophagy might 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 high incidence of liver cancer (55). DR increases Sod1^{-/-} mice lifespan to levels comparable to wild type mice fed ad libitum. The reduced death in Sod1^{-/-} mice under DR results from decreases in both neoplastic and non-neoplastic disease. 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 dietary restricted Sod1^{-/-} mice (56). Moreover, the muscle atrophy observed in Sod1^{-/-} mice fed ad libitum is attenuated by DR. Sod^{-/-} mice as well as aging mice under DR exhibit better preserved neuromuscular junctions compared to their respective controls, suggesting that the reduction in sarcopenia observed in dietary restricted Sod1^{-/-} mice

might result from maintenance of neuromuscular junction integrity. In summary, DR is a powerful anti-aging 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 might 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, non-myogenic 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 pre-adipogenic cells in aging muscle.

Understanding physiological 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 in longitudinal studies (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 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 multi-center 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 (Caucasian, African-American, Chinese, 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 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 were: 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 mechanism(s) for the ethnic differences in the rates of bone loss?; and 3) 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 whether it 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, effects of sex steroid deficiency on bone are reversed by antioxidants (67). Age-related activation of PPAR γ by ligands generated from free fatty acid oxidation also leads to an attenuation of Wnt signaling and 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 VEGF 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- 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 TNF (71,72), a cytokine which 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 M-CSF, RANKL, and OPG. Following ovariectomy, activated T-cells home preferentially near endosteal surfaces to support the nearby formation of osteoclasts. Recent studies have also shown that ovariectomy leads to an accumulation of ROS, which then leads to an expansion of T cells due to increased presentation to T-cells of antigen fragments bound to MHC molecules expressed on antigen presenting cells (macrophages and dendritic cells). Key research questions identified included: 1) Do T-cells play a significant causal role in postmenopausal osteoporosis or in other forms of bone loss (e.g., primary hyperparathyroidism, steroid osteoporosis) in humans; 2) Does estrogen

deficiency cause a non-specific 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 central nervous system (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-I, and cortisol production, each of which modulate 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 physiological 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.

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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. Uitterlinden, Erasmus University, Rotterdam, 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, 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 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, The 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, Bethesda, MD; *Understanding Physiological 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, AR; Roberto Pacifici, Emory University School of Medicine, Atlanta, GA; Edith M. Gardiner, University of Washington, Seattle, WA; Thomas L. Jernigan, Johns Hopkins University, Baltimore, MD; Jane B. Lian, University of Massachusetts Medical School, Worcester, MA.

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