NICHD Conference Abstracts: Determinants of Peak Bone Mass

Peak Bone Mass: When is it achieved?

Babette Zemel, PhD

Peak bone mass (PBM) is has been defined as: (1) the maximum bone mass attained in adulthood; (2) the amount of bone acquired when bone gain ceases; and the BMD during the plateau that follows growth and bone mass accrual and precedes the bone loss that occurs later in adulthood. These definitions lead to different approaches for identifying PBM. Most commonly, PBM has been defined based on cross-sectional studies. However, this approach fails to determine when bone accretion ceases at the individual level. Peak gains in bone mass occur about 6 months after the adolescent growth spurt in height, but gains in total bone mineral content continue for years after that point. Only a few studies have considered the gains that occur in late adolescence and into adulthood. A prospective longitudinal study of Swedish ~800 military recruits showed that between 18 and 24 years of age, gain in BMD were related to the timing of adolescent growth spurt, and to changes in physical activity over a 5 year period. The Canadian Multicentre Osteoporosis Study (Berger et al. 2010) demonstrated differences between males and females by skeletal site in the timing of PBM. Lastly, examination of longitudinal changes in cortical and trabecular bone (Riggs et al. 2008) show very different trajectories of PBM for different bone compartments. Overall, these studies demonstrate that estimates of PBM vary depending on study design, skeletal site, the bone outcome measured and methodology used, sex, population ancestry, maturational timing, and physical activity in young adulthood. Characterizing the development of PBM is central to understanding skeletal biology and the human life cycle, and has clinical implications diagnosing osteoporosis in adulthood. Understanding the long term health implications of timing and magnitude of PBM is important for public health initiatives and clinical decisions.

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Gender differences in bone accrual and structure

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A top priority in osteoporosis research is the identification of the structural basis and the genetic factors that contribute to variations in the risk for fragility fractures. The main areas of progress in osteoporosis research during the last two to three decades have been: (1) the general recognition that this condition (which is the cause of so much pain in the elderly) has its roots in childhood and (2) the identification of the structural basis accounting for much of the variations in bone strength among humans.

Progress in elucidating the structural basis for sex differences in the prevalence of osteoporosis – a disease characterized by low bone mass and the development of non-traumatic fractures – has been considerably greater for the axial skeleton than for the appendicular skeleton. Accumulating evidence suggests that a diminished accrual of bone in girls is the basis for the lower peak bone mass (PBM) in young women, which, in turn, is a major determinant of their two- to fourfold higher incidence of vertebral fractures when compared to men. Available data also indicate that sex differences in PBM in the axial skeleton are the consequence of differences in vertebral growth, rather than in bone density (Gilsanz et al. *Radiology,* 1994). The cross-sectional area (CSA) of the lumbar vertebrae is 25% smaller in young women than in men, even after accounting for body size. This disparity is also present in children and has most recently been found to be present as early as birth; newborn girls have, on average, 10.6% smaller vertebral cross-sectional dimensions when compared to newborn boys. (Ponrartana et al. *J Pediatr*, 2015).

Since the CSA of the vertebral body is a major determinant of its compressive strength, the smaller vertebral CSA of females imparts a mechanical disadvantage that increases the stress within the vertebrae for all physical activities, and if persists, the susceptibility for fragility fractures later in life. The smaller female vertebral CSA also results in greater flexion/extension and lateral flexion. Because greater flexibility of the spine may facilitate the lordosis needed to maintain upright posture, it could be hypothesized that fetal load is a selection factor in the evolution of the discrepant spinal morphology between the sexes in humans.

While current knowledge suggests that the predisposition for osteoporosis may be present at birth, they do not preclude the notion that bone growth can be optimized as a result of simple mechanical interventions. Hence, the need for preventive strategies to start in early childhood and be geared toward those infants with the smallest vertebrae and highest risk for developing vertebral fractures later in life.

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Bone Strength Accrual

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Bone strength is more directly related to fracture risk than bone mass, density, or other common bone measures. This is because fractures occur when loading exceeds bone strength. Strength (failure load) is a structural property that depends on both geometric properties (size, shape) and material properties (density, modulus). Strength differs depending on the type of loading applied (e.g., compression, bending, torsion), and the properties contributing most to fracture risk differ depending on the skeletal site. Since bone strength must be evaluated noninvasively for clinical purposes, it is usually assessed using imaging techniques including dual-energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT), magnetic resonance imaging (MRI), and quantitative ultrasound (QUS). Each modality assesses different bone properties, and all have their own advantages and disadvantages. It should be noted that 3-dimensional imaging is needed to accurately assess many of the properties of interest.

In the axial skeleton, the primary concern is resistance to compressive loading. The main properties of interest include cross-sectional area, cancellous density, and total mass of the vertebral body. During growth, vertebral cancellous density is stable in both boys and girls prior to puberty, increasing significantly between the ages of 12-17 years for boys and 10-15 years for girls (Gilsanz et al. 2009). Density is similar for black and white children through Tanner stage 3, but diverges with higher density in blacks at Tanner stages 4 and 5 (Gilsanz 1999). Vertebral cross-sectional area increases throughout growth and is larger in males than in females of similar stature at all ages, even at birth. No differences in vertebral cross-sectional area are seen based on race.

In long bones of the appendicular skeleton, there are different strength considerations for the diaphysis and metaphysis. Since the diaphysis is comprised of primarily cortical bone in a cylindrical structure, geometric properties such as cross-sectional area, cortical bone area, cortical thickness, and periosteal and endosteal circumference dictate strength. Structural measures such as moments of inertia, section modulus, and stress-strain index are often calculated based on bone geometry as surrogate measures of strength. Cortical bone area at the midshaft of the femur increases during growth in correlation with anthropometric measures, especially weight. These changes do not appear to differ based on race or sex (Moro 1996). Because the metaphysis undergoes significant changes in morphology during growth, it is less clear what measures best characterize strength in this region. Cancellous density, cross-sectional area, total mass, and stress-strain index have been used at specific locations relative to bone length or averaged across the entire metaphysis. In contrast to the axial skeleton, appendicular bone properties do not peak since the periosteum and endosteum continue to expand throughout life.

While changes in axial and appendicular geometric and material properties during normal development are relatively well understood, much less is known about possible deficiencies in bone accrual in vulnerable patient populations, particularly those with decreased physical activity and loading of the skeleton due to mobility impairments or immobilization. Research is needed

to understand the magnitude and type of bone deficits typical of different patient populations both to mitigate short term fracture risk and to understand long term risks as these patients age.

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Research Gaps

1. Understanding the relative contributions of bone size, geometry, density, and microarchitecture to bone strength at different skeletal sites in different pediatric populations.
2. Understanding the magnitude and main contributors to deficient bone strength accrual in vulnerable populations such as children with neuromuscular disorders.
3. Understanding how different interventions (mechanical and pharmaceutical) affect bone structure and strength in children at risk for deficient bone accrual.

Fracture Risk and Tracking

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The frequency of fractures is higher among children compared to young and middle-aged adults, reflecting the vulnerability of the growing skeleton prior to peak bone mass attainment. The skeleton is particularly vulnerable to fracture during the period of rapid growth in late childhood and early adolescence, when linear bone growth may outpace bone mineralization resulting in a further transient increase in bone fragility. Among healthy children, approximately one-half of boys and one-third of girls will sustain a fracture by age 18 y, with one-fifth sustaining two or more fractures. Boys and girls of European ancestry have a greater fracture risk than children of African ancestry. The most common site of childhood fracture is the forearm resulting from falls to an outstretched arm. Although physical activity is critical for bone modeling, children with higher levels of physical activity are more likely to fall and sustain a fracture.

Bone characteristics during childhood and adolescence are associated with fracture risk in the short term, and optimizing bone accrual during growth is important for prevention fractures. Children with a forearm fracture are more likely to have lower bone mass and areal bone mineral density (aBMD) by dual-energy x-ray absorptiometry (DXA) and volumetric BMD (vBMD), cortical area, and bone strength by peripheral quantitative computed tomography (pQCT). A one standard deviation decrease in bone mass or density has been associated with a 40% to 89% increase in fracture risk. Moreover, among children who experience similar forearm injuries, those with lower bone density were more likely to fracture. Notably, children with a forearm fracture have deficits in bone mineral throughout the skeleton not just at the forearm. Studies

using high-resolution pQCT (HRpQCT) have revealed micro-architectural changes that underlie increased bone fragility in children who sustain a forearm fracture following mild trauma, such as thinner cortical bone, lower total vBMD, and increased cortical porosity, particularly in boys.

Whether bone status (i.e., bone mass or strength relative to one's peers of the same age and sex) during childhood is associated with osteoporotic fractures in the elder years remains speculative. Our best approximation comes from examining the association between bone status during different periods of growth and peak bone mass attained in early adulthood. In other words, does bone status "track" throughout growth to skeletal maturity? Tracking refers to the stability or continuity of a measure over time. The degree to which bone status tracks from childhood to young adulthood and beyond is of critical importance to the concept of optimizing peak bone mass for lifelong skeletal health. Numerous studies have demonstrated that measures of bone status track quite strongly from childhood through adolescence, with most tracking correlations falling in the range of 0.6 to 0.7 depending on the skeletal site, trait, and duration of follow-up. Tracking correlations transiently decline during early adolescence, likely due to variability in the timing of puberty. These findings provide strong evidence that bone status during childhood is indicative of peak bone mass. However, the fact that tracking correlations are far from unity suggests that lifestyle factors can alter bone status in both positive and negative directions. Indeed, deviation from tracking has been associated with sports participation, and changes in relative growth and adiposity. Identification of factors that positively or negatively influence tracking, or an individuals bone accrual trajectory, provides insights to interventions to optimize peak bone mass. Identification of individuals who are most likely to benefit from such interventions will inform practical implementation strategies.

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Research Questions

1. Are fractures in childhood predictive of osteoporotic fractures in older adulthood?
2. To what extent is tracking of bone mineral status a reflection of genetic endowment vs. a relative consistency in behaviors?
3. To what extent can childhood bone status measures predict low peak bone mass and what implications does this have for screening?

Genetic Determinants of Bone Mass

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Bone mineral density (BMD) is a classic example of a complex trait influenced by behavioural, environmental and genetic factors. There is strong evidence for a genetic component to BMD determination and in the predisposition of the clinical endpoint of osteoporosis, with an estimated 60-80% of the variability in the risk explained by heritable factors. Uncovering the genetic architecture of bone mass has implications for both therapeutic and diagnostic opportunities.

The first genetic discoveries related to BMD used the candidate gene approach. Key loci from that era include a variant in the regulatory region of the collagen 1 alpha 1 (*COL1A1*) gene[1,2], and variation in the vitamin D receptor (*VDR*) gene[3]. Subsequent genome-wide linkage scans in families allowed for the first non-hypothesis driven genome wide appraisals, albeit with relatively low resolution. One of the most notable linkage reports was with a region on chromosome 11q12-13 in a kindred with autosomal dominant inherited high bone mass, leading to the discovery of a key mutation in the 'low-density lipoprotein receptor-related protein 5' (*LRP5*) gene[4].

The relatively contemporary genome wide association study (GWAS) approach is a more comprehensive and unbiased strategy to identify causal genes related to complex phenotypes. Larger and larger GWAS efforts have uncovered an increasing number of loci contributing to bone mass related traits, with the latest meta-analysis reporting 56 adult BMD and 14 fracture risk associated signals[5].

Whole genome sequencing has been a recent advance to the genome-wide approach repertoire. The advent of such technology now enables the analysis of candidate genes and pathways, as well as unbiased approaches. One of the more impressive examples of this methodology includes the uncovering of rare variants near the 'engrailed homeobox 1' (*EN1*) gene and their association with areal BMD in a large sample of European adults[6].

There is also overwhelming evidence that genetic factors play a role in bone accretion and strength during growth in early life. Identifying the factors that influence bone acquisition during childhood in order to achieve optimal adult peak bone density has important implications for prevention of osteoporosis in later life. Indeed, working with the NICHD Bone Mineral Density in Childhood Study (BMDCS), we have reported loci that influence pediatric bone density, content and maturation at various skeletal sites[7,8].

The uncovering of the genetic architecture of bone mass has implications for both novel therapeutic and diagnostic approaches, but it is clear there is still much to learn about the genomic architecture underpinning the pathogenesis of osteoporosis.

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Endocrine Aspects of Linear Growth and Epiphyseal Closure

Ron Rosenfeld

Despite the fact that human growth is a quantitative trait that is easily measured and has a high degree of hereditability, it is a remarkably complex biological process. Genome-wide association studies have already identified over 400 single nucleotide polymorphisms that collectively account for ~20% of the variability in adult height. Additionally, adult height is influenced by both intrauterine and postnatal growth, as well as the timing of puberty and epiphyseal closure. Human height velocity curves demonstrate features that distinguish homo sapiens from other mammals: 1) maximal growth rates occur during gestation, with deceleration after birth; 2) relatively late sexual maturation, accompanied by a pronounced adolescent growth spurt; and 3) a delay between puberty and reproductive capability.

The combination of animal knock-out studies and human mutational analysis has convincingly demonstrated that ~2/3 of prenatal and postnatal growth is attributable to the growth hormone (GH) – insulin-like growth factor (IGF) system. Remarkably, while intrauterine growth is strongly IGF-dependent, it is independent of GH secretion and action. At birth, IGF-I production becomes profoundly dependent upon GH binding to its dimeric receptor and activation of the STAT5b pathway, which results in IGF-I gene transcription.

The pronounced adolescent growth spurt characteristic of both males and females coincides with the production of sex steroids during puberty. It is of note, however, that puberty is also marked

by dramatic increases in GH and IGF-I secretion, reaching levels which would be considered "acromegalic" in the adult. Ultimately, epiphyseal fusion, which can be directly attributed to estrogen action, limits adult height, although mild "shrinkage" has been documented to occur after epiphyseal closure, presumably reflecting stabilization of the growth plate.

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Research Gaps

1. What is the "molecular switch" which leads to GH-dependence of IGF-I production at birth?
2. What are the relative roles of androgens, estrogens and IGFs in the adolescent growth spurt?
3. How well do serum concentrations of IGF peptides reflect tissue levels?

Bone as an Endocrine Organ

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Several cell biological and clinical observations concur to suggest that bone physiology is intimately linked to energy metabolism and fertility. This led us to propose that that there is a coordinated regulation, endocrine in nature, of bone mass, energy metabolism and fertility. We have over the years explored all facets of this hypothesis. The most far reaching implication of this hypothesis is that bone should be an endocrine organ regulating these two physiological processes. Accordingly, we have shown that the most abundant non collageneous protein of the bone matrix, osteocalcin, is in fact an hormone regulating insulin synthesis by pancreatic B-cells and glucose homeostasis, testosterone synthesis by Leydig cells of the testes and male fertility. We have also shown that osteocalcin achieves these functions after binding to its cognate receptor, a GPCR called Gprc6a. However, osteocalcin does more than regulating these two important physiological functions. Indeed osteocalcin is necessary for brain development and for memory in adult mice. The presentation will review the reasoning leading to suspect that bone maybe an endocrine organ, what is already known about the physiological functions of osteocalcin and some of the issues that need to be addressed.

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Research Gaps

1. Do we know all the physiological functions regulated by osteocalcin?
2. What is the nature of the receptor mediating osteocalcin functions in the brain?

3. Can Osteocalcin improve physiological functions it regulates in wild- type mice?

Effects of Maternal Obesity on Fetal Bone Development

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Nutritional status during intrauterine and early postnatal life impacts the risk of chronic diseases, presumably via epigenetic mechanisms (1, 2). Recent evidence shows that maternal Western dietary pattern is associated with significantly increased risk of childhood fractures in offspring (3), however, the impact of gestational events on regulation of fetal bone development is still understudied. Here, we designed studies both in rodents and humans to investigate the effects of maternal obesity on bone development. First, female Sprague-Dawley rats were fed either a low-fat AIN-93G control diet or a high fat diet (HFD) (45% fat calories) for 10 wks starting at 6 wks of age. After 10 wks of these diets, lean (from control diet) and obese (from HFD) female rats were time-impregnated (n=6 per group) by control diet male rats. At gestational day 18.5 (E18.5), all fetuses were taken and embryonic osteogenic calvarial cells (EOCCs) were isolated. We found epigenetic regulation of polycomb-regulated genes in embryonic rat from HFD obese dams. Increased enrichment of repressive histone mark H3K27me3 on the gene bodies of IGF1 and TCFAP2 was synergized with decreased H3K27me3 at the promoter and gene body of BMP4 in EOCCs from HFD obese dams. P53/p21-mediated increase of cell senescence signaling and decreased aerobic glycolysis was imprinted in HFD-EOCCs resulting in decreased cell proliferation and decreased osteoblast differentiation potential. Second, twenty four pregnant women [12 obese (pre-pregnancy BMI≥30 Kg/m^2) and 12 lean (pre-pregnancy BMI 19 - 25 Kg/m^2)] were recruited during last three years. Upon delivery, placentas and umbilical cords (UCs) were collected and cells from the UC matrix were isolated as UC human mesenchymal stem cells (UC MSCs). The UC MSCs were counted and plated in growth media in a single well of a six-well plate. Cells were expanded until the third passage after which they were fluorescently labeled with antibodies against CD13, CD29, CD44, CD90, CD105, CD31, CD34, and CD45 and analyzed via FACS. The UC MSCs of obese mothers displayed less potential toward osteoblastogenesis and more toward adipogenesis. These human MSCs and placentas from obese mothers not only exhibited increased cell senescence signaling and decreased glucose metabolism but also insulin resistance. These findings indicate fetal pre-osteoblastic cell senescence signaling and glucose metabolism programming by maternal obesity in both rodents and humans. *This work was funded by USDA-ARS Project 6026-51000-010-05S.*

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Evidence Based Review of Nutrition and Physical Activity

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The National Osteoporosis Foundation commissioned a writing group of experts in pediatric bone to update a previous statement on factors that affect development of peak bone mass (1). This extensive, updated review (2) included developmental and timing aspects of accruing bone mass, strength of methodological approaches to the study of the phenomenon, lifestyle and environmental factors affecting bone accrual, a discussion of research gaps, and an implementation strategy for improving bone health in children. A systematic review of the literature between 2000, the date of the previous review (1), and January 2015 on 18 factors in relation to bone mass accrual was conducted. One published system of assigning an evidence grade to each factor was applied to differentiate the evidence of each of the 18 factors as shown in the table below.

Lifestyle Factor	**Grade**
Macronutrients	
• Fat	D
• Protein	C
Micronutrients	
• Calcium	A
• Vitamin D	B
• Micronutrients other than calcium and vitamin D	D
Food Patterns	
• Dairy	B
• Fiber	C
• Fruits and vegetables	C
• Detriment of cola and caffeinated beverages	C
Infant Nutrition	
• Duration of breastfeeding	D
• Breastfeeding versus formula feeding	D
• Enriched formula	D

Lifestyle Factor	Grade
Adolescent Special Issues	
• Detriment of oral contraceptives	D
• Detriment of DMPA Injections	C
• Detriment of okWg	C
• Detriment of alcohol	D
Physical Activity and Exercise	
• Effect on bone mass and density	A
• Effect on bone structural outcomes	B

Such an approach has many limitations including lack of consistent bone outcome measures, poor ability to assess exposures, and common issues with the highest level of evidence (the randomized, controlled trial) including small sample sizes, diverse intervention doses, short study durations, variable baseline status, and poor retention and compliance. Despite these limitations, calcium and physical activity were graded level A. Knowing the limitations of systematic reviews, we also reviewed known functional roles of each factor. For example, if an essential nutrient is a constituent of bone, it is required in the diet for bone accrual. A short-term trial of a nutrient supplement may or may not increase bone accrual depending on the status of the nutrient/adequacy of the diet, but it does not disprove the essentiality of the nutrient.

An interesting observation was made as nutrition and physical activity experts worked together (3). Nutritionists traditionally take a reductionist approach and evaluate the effects of a single nutrient with much less effort placed on diet patterns. In contrast, physical activity specialists generalize all forms of physical activity with less effort given to comparing the type, duration, and mode of exercise on bone accrual.

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Research Gaps

1. Better biomarkers of exposure – diet, physical activity, environmental regulators, alcohol
2. Optimal or reference range of regulators of bone accrual – PTH, FGF23, sclerostin, IGF-1, etc.
3. Life stages of growth for effective interventions

Vitamin D Effects on Bone Development

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The primary function of calcitriol, the hormonally active metabolite of vitamin D, is calcium regulation and maintenance of skeletal mass primarily through effects on calcium absorption. However, calcitriol is also hypothesized to influence bone indirectly, i.e., through influences on muscle function. The efficacy of vitamin D to augment bone mineral accrual and bone strength in children has received heightened attention over the past 15 years. Since the year 2000, eight randomized controlled trials (RCTs) were conducted resulting in nine publications. Five of these publications reported beneficial effects of vitamin D on bone outcomes, while the remaining four described null findings. The trial with the most compelling results showed that Lebanese girls who received weekly doses of 14,000 IU vitamin D_3 (high dose) for 12 months had greater gains in hip bone mineral content (BMC) gains compared to placebo (12.8% vs. 7.8%)[1] and a decreased buckling ratio of the femoral narrow neck (-6.6, -4.2 and -1.9 in the low dose, high dose and placebo, respectively). However, there was no vitamin D effect on bone outcomes in the boys or postmenarcheal girls. The other RCTs displaying positive results showed gains in BMC at the femur, lumbar spine and total body with vitamin D supplementation, though two of these studies made their determination based on compliance-based analyses. The null findings reported in the four RCTs might be attributed in part to differences in populations studied, small sample size, low vitamin D intervention doses, and/or baseline serum 25(OH)D concentrations above a threshold necessary to elicit an effect. The overall evidence rating for vitamin D was moderate evidence "B." The evidence grade reflects the lack of generalizability across each RCT, which included primarily female subjects with little diversity in population ancestry. Gains in bone mass resulting from vitamin D supplementation are unlikely coupled to increased calcium absorption, as a recent dose-response RCT showed no effects of vitamin D supplementation on fractional calcium absorption in children with serum 25(OH)D > 50 nmol/L.[2] However, the beneficial effects of vitamin D supplementation on bone are likely enhanced among participants with low baseline 25(OH)D concentrations. The mechanism responsible for the bone response to vitamin D supplementation needs to be investigated further. It is unclear to what extent the developing skeleton's response to vitamin D supplementation differs by sex, population ancestry, stage of maturation, vitamin D dosage, baseline serum 25(OH)D, body composition, and physical activity level. Future RCTs should be designed to address these factors.

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Research Gaps

1. Is there a serum vitamin D threshold associated with a positive bone response to supplementation?
2. Does the bone response to vitamin D differ by maturational stage, sex and population ancestry?
3. Are there vitamin D interactions with other nutrients and or physical activity?

Evidence of the contribution of calcium nutrition to bone accrual

Joan Lappe, Ph.D.

The skeleton contains >99% of the body's calcium and serves as a functional reserve to offset shortages of calcium needed for homeostasis. Bone mineral is 40% calcium, and the source of that calcium is dietary intake. The calcium reserve is very large relative to the cellular and extracellular metabolic pools of calcium; thus, dietary insufficiency rarely impairs calcium-dependent biochemical functions. However, long-term deficiency depletes the reserve and impairs bone mass and strength. Because the human skeleton contains only about 2%–3% of the total adult body calcium at birth, the dietary requirements for calcium during the first 20–30 years of life are determined primarily by skeletal growth. Calcium is a threshold nutrient, and balance studies have guided recommendations for the threshold of calcium intake for children and adolescents by age and sex. However, surveys show that not all children achieve optimal calcium intake, and calcium intake is poorer, on average, in children and adolescents at the very time their bone mass is increasing the fastest.

Numerous studies have been conducted to determine the amount/types of dietary calcium needed for development of optimal bone mass and strength and the ages/stages of development at which calcium intake might be more critical. Furthermore, efforts have been made to elucidate the relationship between calcium intake and physical activity in maximizing skeletal development.

The search for calcium studies identified 16 RCTs published since 2000, encompassing 3077 individuals. All reported baseline calcium intakes were below the Institute of Medicine recommended levels. The calcium interventions varied from supplementation with pills/chews to use of calcium-fortified foods or dairy foods. The calcium intervention doses ranged from 500 to 1200 mg/d. In these calcium-deficient populations, 90% of the RCT's detected a statistically and biologically significant effect on bone accrual. We found that calcium supplementation, whether with pills, fortified foods, or dairy, consistently increases gain in skeletal mass and density measures in children and adolescents, usually between 1.0% and 5.0%. Four of the five RCT's of calcium and exercise combined found that the combined intervention had a significantly greater effect on bone accrual than either exercise or calcium alone, and one also found an interaction effect on cortical thickness and area as measured by pQCT.

The skeletal sites showing a calcium effect were widely varied among the studies. Most of the studies included primarily white subjects. Most studies evaluated the effects of calcium intake on

DXA outcomes, including BMC, aBMD, and bone area of the total body, lumbar spine, total hip, femoral neck, intertrochanteric and trochanteric areas of the hip, and distal and ultradistal areas of the forearm. Very few studies reported all possible DXA outcomes, and specific outcomes varied among studies. Three RCTs assessed bone mass and structure using pQCT.

Our evidence grade of **A** for calcium was based on findings that multiple RCT's show a positive effect on ***at least one*** skeletal measure when average baseline intake is below the estimated average requirement. The bone accrual in the supplemented group compared to the placebo group was largest in children who had the lowest intakes at baseline, which is to be expected with a threshold nutrient such as calcium. We designated a grade of **B** for dairy based on the findings that two out of three RCT's show positive effects on bone accrual.

Gaps in the research include determining the combination/s of calcium intake and physical activity are most effective in increasing bone accrual; the effective ways of modifying dietary behavior in children and adolescents so that they habitually consume recommended levels of calcium; and whether bone accrual in response to calcium supplementation varies by population ancestry.

Effects of Nutritional Deprivation on Bone

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Anorexia Nervosa (AN) is a psychiatric illness with profound medical consequences.

This lecture will review complications associated with eating disorders, with a focus on skeletal sequalae. Endocrine alterations will be reviewed, as well as other factors that contribute to diminished bone mass and skeletal strength in this population. Optimal screening and treatment recommendations will be considered, considering the evidence that drives current clinical practice. Gaps in knowledge will be highlighted that drive a future research agenda. As will be discussed, the overarching goal is the identification of strategies to maximize peak bone mass in this patient group and prevent osteoporosis later in their lives

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Hormonal Contraception and Bone

Madhusmita Misra, MD, MPH

More than 60% of women of reproductive age use some form of contraception and about 40% of these women use hormonal methods. The latter include progesterone only and estrogen-progesterone combination contraception.

Of the progesterone only methods, those that do not impact ovulation or estrogen levels are typically not associated with deleterious effects on bone. In contrast, depot medroxy-progesterone acetate (DMPA), which inhibits ovulation and reduces estrogen levels, causes marked reductions in bone density, particularly in the first few of years of use, following which bone loss stabilizes. Bone losses appear to be greater in adolescent girls than in older women over a similar duration; however, recovery occurs quickly following DMPA discontinuation, particularly for the spine. While a black box warning exists regarding the impact of DMPA on bone, guidelines clearly indicate that its use should not be restricted for such concerns, particularly in women 18-45 years old, because the benefits of pregnancy prevention likely exceed any deleterious effects of DMPA on bone. Of note, data are currently lacking regarding the impact of prolonged DMPA use in adolescents on peak bone mass acquisition. Studies suggest a 17--50% increase in fracture risk in current or past users of DMPA, with a higher risk of fracture observed with longer duration of use, although factors inherent to DMPA users likely also contribute to this risk. It is still unclear whether a long duration of DMPA use starting before peak bone mass acquisition leads to higher lifetime fracture risk compared to a similar duration of DMPA use starting after peak bone mass acquisition. A few studies have examined the impact of norethisterone injections and the levonorgestrel subdermal implant on bone, and available data are conflicting. The levonorgestrel IUD and vaginal ring, and progesterone only oral pills do not appear to cause reductions in bone density.

Estrogen-progesterone combination methods include the combined oral contraceptive pill (COC), the transdermal contraceptive patch (TDC) and the contraceptive vaginal ring (CVR). Concerns have been expressed regarding the impact of COCs on bone, particularly those containing very low doses of estrogen. In studies in adolescents that compared the effect of COCs to DMPA and no hormonal contraception, effects of COCs on bone were typically intermediate. While these data are relatively reassuring, they are also concerning in that pubertal bone accrual is impacted at least to some extent by this form of contraception. In fact, cross sectional and prospective fracture studies suggest as much as a 30% higher risk of fracture with COC use, particularly in younger women, whereas COC use appears to be neutral or even protective against fracture risk in older women. The suboptimal bone accrual observed in young COC users has been attributed to its hepatic first pass effects leading to IGF-I suppression, an important bone trophic factor (particularly in adolescents), and to reductions in testosterone from ovulation suppression (testosterone being bone anabolic with anti-resorptive effects). Of note, limited data are currently available regarding the impact of TDC or CVR on bone. Estrogen delivered by both systems bypasses hepatic first pass metabolism, and does not suppress IGF-I. More long-term studies are necessary to examine the impact of these measures on bone health when used before versus after peak bone mass acquisition.

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Effects of Glucocorticoids on Bone

Mary Leonard, M.D.

Glucocorticoids (GCs) are highly effective and widely prescribed for the treatment of myriad childhood diseases; however, they have adverse effects on osteoblasts, osteocytes, and osteoclasts. It is well established that administration of GCs results in significant reductions in bone formation due to decreased osteoblast differentiation, function and lifespan. GCs also increase osteocyte apoptosis and osteocyte-derived wnt/ -catenin antagonists (e.g. DKK-1). GC therapy results in an early and transient increase in bone resorption due to enhanced osteoclastogenesis, followed by abnormalities in the cytoskeleton that prevent bone resorption. The growing skeleton is uniquely vulnerable to the effects of GCs on bone formation and recent longitudinal studies have demonstrated that greater glucocorticoid exposure in children is associated with incident vertebral fractures. Importantly, studies of glucocorticoid effects on bone accrual are confounded by the impact of the underlying inflammatory disease. Cytokines, such as TNF-, have direct adverse effects on osteoblasts that are strikingly similar to GC effects. In contrast, cytokines result in sustained increases in bone resorption.

Persistent inflammation and elevated cytokine levels characterize childhood diseases treated with chronic GCs such as inflammatory bowel disease and juvenile idiopathic arthritis. In contrast, steroid sensitive nephrotic syndrome (SSNS) responds promptly and completely to GC therapy, and the nephrotic state is quiescent during high-dose therapy. Unfortunately, SSNS relapses in the majority of children when the GC dose is reduced, resulting in protracted and repeated courses of therapy. Therefore, SSNS serves as a clinical model, with minimal systemic inflammation, to examine the independent effects of GCs on BMD and cortical structure during growth. A pQCT study in children and adolescents with SSNS demonstrated significantly lower trabecular BMD and higher cortical BMD, respectively, compared with healthy children (Wetzsteon, et al. J Bone Miner Res 2009). The authors attributed the elevated cortical BMD to lower bone formation and greater secondary mineralization, a hypothesis that was supported by findings from their subsequent longitudinal follow-up study: greater GC dose, lesser increases in tibia length and lesser increases in cortical area were significantly and independently associated with greater increases in cortical BMD in SSNS (Tsampalieros, et al. J Bone Miner Res 2013). Additional studies confirmed an association between GC exposure and increased cortical BMD in children with varied chronic diseases.

Compared with their healthy peers, children with SSNS had elevated BMI and greater fat area (by pQCT), consistent with GC effects. In turn, similar to obese healthy children, children with SSNS had greater muscle area, which was associated with greater periosteal circumference at the tibial shaft. However, when these relationship were explored in the follow-up study, gains in tibia length were associated with declines in periosteal circumference Z-scores in children treated with GCs for SSNS. These data suggest that the greater muscle mass (secondary to greater fat mass) in children treated with GCs for SSNS promoted bone accrual, but GCs prevented normal gains in periosteal circumference during growth. Although GCs are known to impair muscle metabolism, a subsequent analysis in these SSNS patients demonstrated normal muscle strength relative to muscle area (Lee, et al. J Bone Miner Res 2015). A similar longitudinal study conducted in children with Crohn's disease demonstrated that greater glucocorticoid exposure was associated with impaired periosteal bone accrual during linear growth, independent of clinical disease activity (Tsampalieros, et al. JCEM 2013). Finally, studies in children with leukemia demonstrated rapid gains in trabecular BMD and periosteal circumference following completion of GC therapy (Mostoufi-Moab, et al. JCEM 2012).

Taken together, these studies suggest that GC effects to decrease bone formation resulted in lower trabecular BMD and impaired periosteal bone accrual. The consistent observation of increased cortical BMD in multiple diseases suggested greater secondary bone mineralization in the setting of relatively lower bone formation during growth.

Research questions:

1. What are the mechanisms and fracture implications of the high cortical BMD observed in children with varied diseases treated with glucocorticoids?
2. What is the impact of anti-resorptive agents on trabecular and cortical BMD and cortical structure in children treated with glucocorticoids?
3. What anabolic therapies can be used to promote trabecular and cortical bone accrual in children treated with glucocorticoids?