IN VIVO PRECISION OF DUAL ENERGY X-RAY ABSORPTIOMETRY LUMBAR SPINE TRABECULAR BONE SCORE IN YOUNG ADULT FEMALES

by

JULIA MADELINE LANCE

(Under the Direction of Connie J. Rogers)

ABSTRACT

Peak bone mass (PBM), attained between 20-30 years of age, influences osteoporosis risk. Trabecular bone score (TBS) is an emerging bone measure that complements clinically-preferred lumbar spine areal bone mineral density (aBMD). This cross-sectional study of 19 females ages 18-30 years examined the in vivo precision of TBS during years surrounding PBM. Spine dual-energy X-ray absorptiometry scans were performed in triplicate according to International Society for Clinical Densitometry guidelines. Percent coefficient of variation (%CV) for spine aBMD and TBS were compared using a paired t-test and relationships between %CV for both measures and body size measures were assessed using Pearson's correlations. Both aBMD (%CV=0.88%) and TBS (%CV=0.89%) were highly precise and did not differ from one another (P=0.51) and measures of body size did not correlate with %CV for aBMD or TBS. In sum, TBS has similar in vivo precision as spine aBMD, which is not confounded by body size.

INDEX WORDS: DUAL ENERGY X-RAY ABSORPTIOMETRY, TRABECULAR BONE SCORE, PRECISION, BONE, YOUNG ADULT FEMALES, PEAK BONE MASS, BONE MINERAL DENSITY, COEFFICIENT OF VARIATION, LEAST SIGNIFICANT CHANGE

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DEDICATION

This work is dedicated to my parents Britton, Tony, Kurk, and Janet for raising, supporting, and loving me throughout my ongoing educational journey, even when my path was uncertain, and my younger brothers: Nate and Justin. I love you both immensely and am so thankful to have you both in my life for laughs, adventures, and fond memories –you both serve as a reminder for me to relax and enjoy life.

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CHAPTER 1

INTRODUCTION

Osteoporosis is a characterized by low bone density, compromised bone microarchitecture, and increased risk for fracture. Osteoporosis, low bone mass, and fragility fractures are major public health concerns that impact >53 million U.S. adults.[1] Despite its prevalence among older adults, osteoporosis has origins in childhood and adolescence, a critical period of skeletal growth and development.[2,3] Around the age of 20 years, bone accrual begins to attenuate, reaching a plateau known as peak bone mass (PBM).[2,3] PBM is defined as an individual obtaining their full genetic potential for bone mass or the point at which bone accrual plateaus.[2] Previous research has established the "tracking" of bone outcomes across the lifespan, indicating that bone outcomes in childhood impact osteoporosis and fragility fracture risk in later years.[3,4] Given this, PBM achieved during young adulthood has been identified as a key determinant of osteoporosis risk.[3]

Traditional bone health assessment measures, such as areal bone mineral density (aBMD, g/cm^2) derived from dual energy X-ray absorptiometry (DXA) currently inform international osteoporosis diagnostic criteria.[5] While aBMD is the gold standard measure for bone health assessment, it fails to assess bone microarchitecture, an indicator of bone quality and an additional determinant of fracture risk.[6] Therefore, Trabecular Bone Score (TBS), an emerging measurement of the lumbar spine (LS) via DXA, was developed as a complementary measure to standard evaluations of aBMD.[6] TBS uses gray-scale texture

analysis to estimate bone microarchitecture of trabecular ("spongy") bone in the LS. [6,7] TBS has been shown to predict fracture risk independently of aBMD and can be incorporated into fracture prediction models to further refine risk estimation.[6,7]

While TBS has been established as a complementary measure to aBMD, the precision of this new measurement has not been determined across the lifespan, particularly within the years surrounding PBM attainment. Determining the precision of TBS is critical in understanding the utility of TBS in monitoring bone health trajectories during the unique period of the adolescent-to-young adult transition, when subtle consolidation of the trabecular bone compartment has been reported.[3] Given this, the current study investigates the precision of TBS and LS aBMD in a sample of healthy young adult females ages 18 to 30 years. In accordance with guidelines published by the International Society for Clinical Densitometry, a precision assessment study was conducted to calculate precision error (percent coefficient of variation; %CV) and internal thresholds (least significant change; LSC) for interpreting measurement error from true biological change in the skeleton.[5] The findings from this study will allow researchers in the University of Georgia's Nutrition and Skeletal Health Laboratory and others to apply the generated LSC values to longitudinal aBMD assessments in young adult females to improve PBM optimization and subsequently, osteoporosis prevention.

The literature review (Chapter 2) provides an overview of the current body of evidence relating to the following topics: 1) bone biology, 2) pediatric bone development, 3) factors impacting bone health, 4) osteoporosis, and 5) bone health assessment methodologies. The final chapter (Chapter 3) presents the cross-sectional precision

assessment study which examines the precision of TBS in healthy young adult females ages 18 to 30 years.

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CHAPTER 2

REVIEW OF THE LITERATURE

Bone

Despite its inert appearance, bone is a living tissue that receives blood supply, consists of multiple cell types, and participates in functions that are essential for living. Bones enable locomotion, protect soft tissues, support the body, and store essential minerals and bone marrow.[1] Bone is comprised of water, organic matrix, and inorganic matrix.[2] Organic matrix accounts for approximately 35% of the total weight of bone tissue and consists primarily of type I collagen fibers.[1-3] Inorganic matrix accounts for the remaining 65% of bone tissue and primarily contains mineralized hydroxyapatite [Ca10(PO4)6(OH)2] which is comprised of phosphate and calcium ions.[1,3] Calcium carbonate, magnesium ions, sodium ions, and trace amounts of potassium, chlorine, and fluorine ions are also found in the inorganic matrix.[1,3] As minerals of the inorganic matrix are deposited within the collagen fibers of the organic matrix, the amount of water in bone tissue decreases.[2] Mineral concentration increases during this process, contributing to increases in bone strength and stiffness.[2]

The human skeleton is comprised of two types of bone tissue: trabecular and cortical. Trabecular bone has a honeycomb-like network of heterogeneous bone lamellar plates and rods which form connections called trabeculae.[4] Trabecular bone is metabolically active tissue, that is highly responsive to mechanical loading and unloading of the skeleton, such as that associated with physical activity (PA) or prolonged bedrest, respectively.[2]

Trabecular bone's sensitivity and ability to adapt to mechanical stress (or lack thereof) is likely due to its relatively large surface area which is related to its porous structure and its immediate access to the vascular system and bone marrow.[2] Trabecular bone is found in the epiphyses/metaphysis (ends) of bones, and axial skeletal sites such as the spinal vertebrae and pelvic bones.[2,3,5] Cortical bone encases trabecular bone and comprises the mid-regions of long bones, forming a dense, compact shell.[2,4] Cortical bone accounts for approximately 80-90% of the mature skeleton and is composed of circle-shaped osteon units.[2,4,6] An interwoven network of blood vessels and nerves supplies oxygen and nutrients to the cortical bone allowing it to respond to stimuli.[2,4] The diaphysis or bone shaft contains thick layers of cortical bone, particularly in long bones such as the femur and humerus to increase stiffness and resistance to mechanical stress.[4]

Modeling and remodeling

To maintain structural integrity and function, bone tissue undergoes the metabolic processes of modeling and remodeling, which are orchestrated by osteoblasts, osteoclasts, and osteocytes.[1,7] Osteoblasts deposit new osteoid tissue on the bone surface while osteoclasts resorb old and damaged tissue on the bone surface via localized acidification and enzyme excretion.[1] Osteocytes are located within the bony matrix of lacunae where they offer structural support and aid in the coordination of bone modeling and remodeling processes through signaling molecules (e.g. receptor activator of nuclear factor kappa beta ligand (RANKL), osteoprotegerin (OPG)).[1,8] Modeling occurs when bone formation (i.e. osteoblast activity) outpaces bone resorption (i.e. osteoclast activity).[7] During childhood and adolescence, modeling results in the longitudinal growth and reshaping of bones in response to mechanical loading of the skeleton, such as that experienced during PA.[5,7] Bone

remodeling refers to the paired processes of bone formation and resorption, in which osteoclast and osteoblast activities are coupled within a temporary bone remodeling unit, termed the "basic multicellular unit" (BMU).[1,5,9] Remodeling is the turnover of bone tissue, which occurs throughout adulthood and renews the skeleton every 10 years.[5] Remodeling aims to maintain bone mass and integrity, adjust bone microarchitecture in response to skeletal loading, repair microdamage, and preserve calcium homeostasis.[1,5,9] The rate of bone resorption increases throughout the lifespan and outpaces bone deposition, resulting in irreversible, progressive losses of cortical and trabecular bone.[5] Despite being a natural symptom of aging, declines in skeletal mass and strength result in fragile bones that are at higher risk of fracture and bone disease.

Pediatric Bone Development

Establishing healthy bones during childhood and adolescence is critical for preventing fracture and bone disease later in life. Osteoporosis, a disease wherein age-related bone loss results in increased bone fragility and fracture risk, has origins in childhood despite its prevalence among older adults.[6,7,10] Bone mass and density increase with longitudinal growth during childhood and adolescence, with a majority of adult skeletal mass being accrued by age 20.[11-14] Generally, bone accrual rates remain modest compared to longitudinal growth during childhood, and peaks 6 months to two years after peak height velocity (PHV) is achieved.[7,13] As pubertal maturation cessation occurs in late adolescence and early adulthood (ages 18-23 years), gains in bone mass begin to plateau, reaching its maximum amount known as PBM (**Figure 1**). PBM is defined as an individual achieving their maximal genetic potential for bone mass, or when increases in bone mass that are experienced during childhood and adolescence attenuate.[6,7] Compared to age-

related declines in bone mass, the accrual, magnitude, and timing of PBM vary widely among individuals.[7] Thus, optimizing an individual's attainment of PBM during the formative years of childhood, adolescence, and young adulthood could mitigate characteristic declines in bone experienced later in life. Computer simulations of bone remodeling predict that a 10% increase in the magnitude of PBM attained during young adulthood could delay the onset of osteoporosis by 13 years for many individuals.[7] Given these findings, researchers hypothesize that PBM could be the most important factor for preventing osteoporosis later in life.

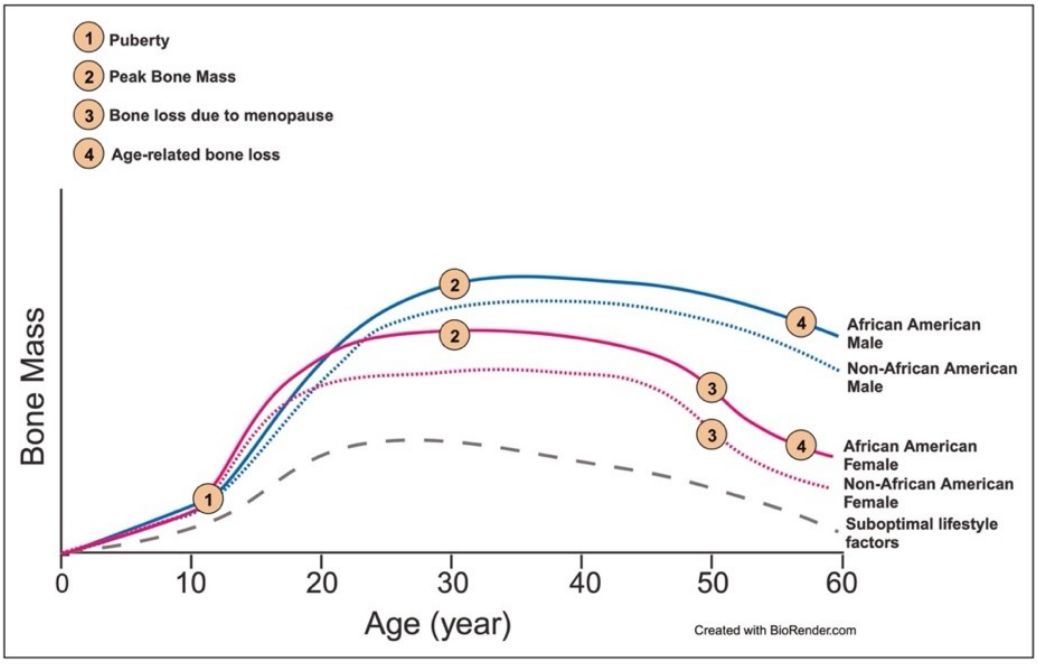

Figure 1: *Bone mass across the lifespan between sex and African American and non-African American ancestry with optimal and suboptimal lifestyle factors (Rodrick and Kindler, 2024).*[6]

Research from the Bone Mineral Density in Childhood Study (BMDCS) has demonstrated that bone mass accrual during childhood is a strong determinant of PBM, and that PBM established during young adulthood influences bone health outcomes later in life.[15] The BMDCS was a mixed longitudinal study of a diverse cohort of healthy children, adolescents, and young adults (n=2,014, 50.7% female; 48% White, 23.8% African American, 17% Hispanic, 11% other races; aged 5-19 years at baseline) recruited from five clinical centers across the U.S.[14,15] From this cohort, >10,000 dual energy X-ray absorptiometry (DXA) measurements were completed and strong correlations between baseline and final spine aBMD measurements were reported after a follow-up time of six years.[15] These findings indicate that bone characteristics "track" across the lifespan, in which individuals with lower (or higher) aBMD measures during childhood and adolescence are likely to have lower (or higher) aBMD during young adulthood.[15,16] Indeed, Wren et al. analyzed a subset of participants from the BMDCS (n=533) who completed the entire six year study duration and reported strong correlations (r=0.66 to 0.87) between baseline and final DXA measurements at multiple scan sites.[17] Furthermore, almost all boys and girls classified as having low bone mass (Z-score <-1.5) at baseline matured into young men and women with lower bone mineral content (BMC; grams (g) of skeletal mass dependent on body size and stature) and aBMD than average (Z-score <0).[17] These findings exemplify the critical role PBM has in determining lifelong bone health, and the importance of optimizing PBM during early years in life to reduce fracture and osteoporosis risk in the future.

As alluded to previously, increases in bone parameters such as BMC and aBMD are most notable during the first two decades of life in which the majority of skeletal mass

and density is achieved. Following the cessation of linear growth, 7-11% of total bone mass is obtained during young adulthood.[7] Findings from Boot et al. report attainment of lumbar spine (LS) and total body PBM between the ages of 18 to 20 years in healthy females and 20 to 23 years in healthy males identifying as Caucasian.[12] Data from the BMDCS provide further insight on PBM attainment and bone accrual in young adulthood. McCormack et al. found site-specific variations in bone mineral accrual and cessation of BMC acquisition.[14] Notably, BMC accrual attenuated and began to decline at skeletal sites rich in trabecular bone (total hip and femoral neck) by age 20 years, particularly among female participants.[14] Whereas cortical bone accrual persisted after cessation of linear growth, as reflected by increases in BMC at the forearm after age 20 years.[14] Prior research from Riggs et al. also reports conservation of cortical bone until the fourth to fifth decade of life, while trabecular bone loss begins in young adulthood and progresses across the lifespan.[18] Furthermore, bone loss during early adulthood may account for approximately 33-50% of total trabecular bone loss over the lifespan.[18] Collectively, these studies are not entirely reflective of the current U.S. population and warrant further investigation among minority groups and individuals of varying health status. However, their findings are providing novel insight on pediatric bone development and revisioning the approach to osteoporosis prevention and treatment.

Factors Impacting Bone Health

Non-modifiable factors

Skeletal growth, maturation, and maintenance across the lifespan are impacted by multiple factors including heredity and genetics, growth and maturation (i.e. puberty), and health behaviors.[7] Heredity and genetics are non-modifiable factors that highly influence

BMC, aBMD, and PBM.[7] Population-based differences in aBMD and other bone health outcomes are evident in scientific literature with Black or African American individuals having the highest aBMD measurements, followed by White or European individuals.[7] Asian and Hispanic individuals have comparatively lower aBMD values.[7] While heredity and genetics are known to greatly impact bone health outcomes, the evolutionary development and expression of these population-based variations remain uncertain.[7]

Growth and maturation profoundly impact bone accrual, specifically during childhood and adolescence.[7,19] BMC and aBMD are both strongly correlated with height during childhood, pubertal maturation, and adolescence.[7] Despite increasing during these critical periods of growth, bone accrual tends to lag compared to gains in height.[7] Bone accrual rates are highest within six months to two years after peak height velocity (PHV) is attained depending on the skeletal site (**Figure 2)**.[7,14] During childhood, sex-based differences in BMC and aBMD are negligible.[7] However, notable differences in BMC, aBMD, and PBM are observed during the latter stages of pubertal development, denoted by breast development in females and genital development in males.[7] Females accrue bone and obtain PBM at earlier ages than males, and generally have comparatively lower BMC, aBMD, and PBM.[7,14,19]

Research has demonstrated these sex- and race-specific variations in the age at which PHV, and subsequently, BMC accrual peak among adolescents.[14] McCormack et al. found girls reach PHV approximately two years earlier on average compared to boys.[14] Additionally, children self-identifying as Black or African American (AA) attained PHV earlier on average compared to non-African American peers (AA girls, 11.0 years, non-

AA girls 11.6 years, $p<.001$; AA boys 13.1 years, non-AA boys, 13.4 years, $p<.001$) (**Figure 2**).[14] Similar sex- and race-specific trends were observed in peak BMC accrual.[14] However, age of peak BMC accrual varied depending on skeletal site (e.g. whole body (WB), lumbar spine (LS), total hip, femoral neck, and forearm measurements) and ranged from an average age of 11.4 to 13.2 years in girls and 13.7 to 14.9 years in boys.[14] Overall, girls experienced PHV and peak bone accrual earlier than boys, and had lower BMC than boys following peak linear growth.[14] Regardless of height similarities, race impacted BMC values, in which children self-identifying as Black or AA had higher BMC values than non-AA peers.[14] These findings implicate that white females had the lowest BMC values, and thus, are at highest risk for fracture and osteoporosis later in life.[14]

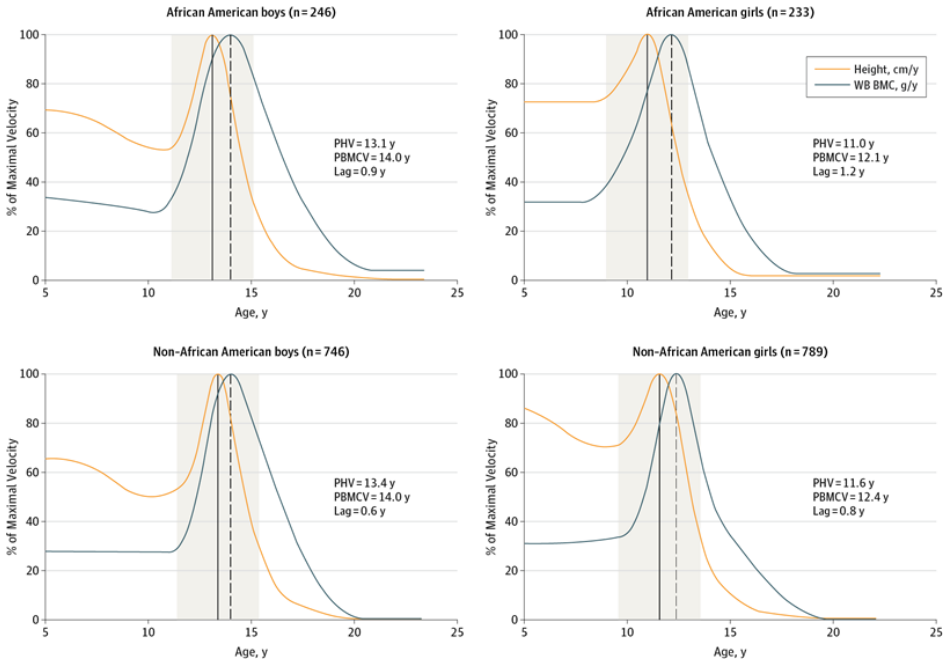

Figure 2: *Relative timing and magnitude of peak height velocity (PHV) and peak whole-body (WB) BMC acquisition, by sex and self-reported race (McCormack et al., 2017).*[14]

Modifiable Factors

Health behaviors including PA, dietary intake, nutritional status, and sleep hygiene are modifiable factors that impact bone. PA plays a critical role in skeletal health throughout the lifespan, and can be considered the most influential modifiable factor for bone health.[7] Bone modeling and remodeling allows bones and various skeletal sites to adapt to mechanical forces imposed by weight-bearing PA (e.g. walking, running, soccer, field sports, gymnastics, plyometric exercises).[7,20] Bone is most responsive to PAs that are dynamic, moderate to high in load magnitude, short in load duration, and are multi-directional in load direction -as opposed to PAs inflicting lower strains at higher frequencies.[7,16,20] Higher strains of mechanical force promote bone modeling, particularly during childhood and adolescence.[7] As such, PA can 1) aid in optimizing bone accrual and PBM attainment during childhood, adolescence, and young adulthood, and 2) minimize age-related aBMD losses throughout the lifespan.[20] A systematic review conducted by the National Osteoporosis Foundation (NOF) identified PA (along with calcium intake) as having the strongest evidence (Grade A) for positive effects on bone outcomes, especially during childhood and adolescence.[16] Of the 20 prospective cohort studies reviewed, statistical differences in BMC or aBMD were reported among 18 studies, demonstrating consistency in findings relating to PA having bone-augmenting effects.[16] The Iowa Bone Development Study (IBDS) and Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) were included in this systematic review. The IBDS reported 10-16% increases in hip BMC and 8% increases in hip aBMD over a 12-year follow-up period, in participants (n=530, 49.6% female; 95% self-identifying as White) who were most physically active compared to those who were less active according to wrist-worn accelerometers.[16,21]

Similarly, a secondary analysis (n=154; 53.2% female; 100% self-identifying as Caucasian) from the larger, mixed-longitudinal PBMAS found that Canadian children who self-reported higher levels of PA during childhood (ages 8-15 years) had 8-10% greater hip BMC as young adults (ages 23-30 years) compared to less active peers (after adjusting for adult PA levels and baseline bone measurements).[16,22]

Additional factors influencing bone strength, such as bone quality and microarchitecture (particularly, that of trabecular bone) can also be enhanced and preserved through osteogenic effects of weight-bearing PA.[20] While there is less robust research supporting the beneficial impacts of PA and exercise on bone structural outcomes (Grade B),[16] researchers have proposed engaging in weight-bearing PA during childhood and adolescence (e.g. gymnastics, resistance training) results in greater and sustained bone strength.[7] These improvements in bone strength were not well maintained in adulthood if PA declines. However, gains in bone mass and strength related to childhood PA remain when comparing bone health outcomes in adults who participated in high levels of PA during childhood to those who did not.[7] Overall, PA is a highly influential factor in maximizing PBM attainment early in life, maintaining bone mass throughout adulthood, and mitigating bone loss during older adulthood.

Dietary intake and nutritional status are additional critical factors for bone health. Micronutrients including calcium, vitamin D, phosphate, zinc, magnesium, vitamin C, and vitamin K contribute to bone accrual during childhood and adolescence.[7] Calcium and vitamin D are of particular importance, given that calcium binds with phosphate to form hydroxyapatite a mineral compound essential for bone structure.[7] Vitamin D aids in calcium absorption and homeostasis in the body.[7] According to the NOF, strong scientific

evidence (Grade A) exists to support the beneficial impacts that adequate calcium intake and supplementation has on bone during childhood and adolsence.[16] Specifically, when studying calcium supplementation via pills/chews, 90% of reviewed randomized controlled trials (RCTs; n=9) found a small, but biologically and statistically significant positive effect on aBMD and/or BMC accrual among children and adolescents.[16] Additional studies investigated calcium supplementation (1,000 mg calcium carbonate/day) among children and adolescents with low calcium intake (<350mg/day) and high calcium intake (≥1,300mg/day), and found greater impacts on bone accrual in children and adolescents with low calcium intake at baseline compared to those that consumed adequate amounts of calcium at baseline.[16] These findings suggest that calcium may be a "threshold nutrient," in which observed benefits on bone accrual during childhood and adolescence may attenuate once the recommended dietary allowance (RDA) of 1,300mg/day is met.[16] However, it is well-known that many U.S. children and adolescents are failing to meet dietary intake guidelines for calcium.[16,23]

Compared to calcium, scientific evidence supporting Vitamin D consumption for bone health was less robust (Grade B) according to the NOF.[16] Four of eight reviewed RCTs investigating Vitamin D supplementation (ranging from 200-300,000 IU of Vitamin D_3) during childhood and adolescence found beneficial effects on bone accrual.[16] While improvements in BMC accrual were reported for ≥50% of considered RCTs, the external validity of findings related to vitamin D supplementation was limited due to largely female sample populations and minimal ancestral diversity.[16]

Dairy, a food group known to contain calcium, vitamin, D, and protein, has also been shown to benefit bone accrual.[16] The NOF reviewed three RCTs investigating dairy

supplementation over a 2-year duration period and found modest, but positive effects on bone outcomes such as tibia cortical thickness and total body aBMD.[16] It should be noted that participants with inadequate (<50%) dairy supplementation did not experience improved bone outcomes, and after supplementation cessation, observed improvements in trochanter BMC were not maintained long-term (>1 year).[16] The NOF assigned the scientific evidence regarding dairy supplementation and PBM a grade of B, given the overall positive effect of dairy consumption on bone accrual and the lack of ancestral diversity in the reviewed RCTs.[16]

In addition to key nutrients or food groups, researchers have considered overall dietary pattern in relation to bone accrual and PBM.[24] The Healthy Eating Index (HEI) is a measure of diet quality that references the Dietary Guidelines for Americans (DGA).[25] In research settings, the HEI can be applied to determine how closely an individual's dietary intake aligns with DGA guidelines by assigning a score ranging from 0 to 100.[24,25] Higher HEI scores reflect higher alliance with the DGA guidelines and are therefore considered "healthier" compared to lower HEI scores.[24] Kindler et al. investigated the relationship between HEI and bone density in youth with healthy weight (n=197), obesity (n=128), and type 2 diabetes mellitus (T2DM; n=90) via a secondary analysis of cross-sectional data.[24] A significant positive association was observed between HEI and aBMD, indicating that youth with higher HEI scores (based on self-reported dietary intake) tended to have greater aBMD measurements (p=0.025 with sex, age, ancestry, and group adjustments).[24] HEI sub-components including "empty calories" (calories from solid fats, alcoholic beverages, and added sugars) and whole grains were also positively associated with aBMD in youth (p=0.010; p=0.052, respectively).[24] Findings from Kindler et al. demonstrate that a diet

aligning closely with DGA guidelines, including whole grains and other high-fiber foods (e.g. fruits and vegetables), and minimizing calories from solid fats, alcoholic beverages, and added sugar is associated with greater bone density in childhood and adolescence.[24]

Aside from dietary intake, nutritional status, such as nutrient deficiencies or being underweight or obese, can negatively impact bone health. Specifically, low body weight (such as that resulting from eating disorders or disease) during childhood and adolescence has been associated with decreased bone density.[7] Obesity has been found to increase bone mass and bone density; however, individuals classified as obese have been found to have an increased risk of fracture.[7] While it is difficult to determine an individual nutrient's impact on bone health, research has found that dietary patterns high in fruits and vegetables resulted in higher rates of bone accrual in children and adolescents.[7] As aforementioned, a diet that is rich in fruits and vegetables, whole grains, dairy, and fiber-rich foods such as legumes, has been associated with improved bone health outcomes and reduced fracture risk during childhood and adolescence.[7] Consuming a diet that contains a variety of foods and supplies adequate amounts of calcium and vitamin D throughout the lifespan is essential for achieving and maintaining optimal bone health.

Despite the established benefits PA and diet can have on PBM accrual, the majority of children and adolescents in the U.S. are failing to meet published PA and dietary guidelines.[25,26] The Physical Activity Guidelines for Americans reports approximately 30% of male high school students and 10-15% of female high school students met aerobic and muscle-strengthening guidelines from 2011 to 2015.[26] Similar trends in PA were observed by Michael et al. when investigating more recent data from the 2019 and 2021 Youth Risk Behavior Survey (YRBS).[27] The YRBS is a cross-sectional, school-based survey which

assesses dietary and PA behaviors among a nationally representative sample of U.S. public and private school students in grades 9-12.[27] According to 2021 YRBS data 31.7% of male high school students and 15.7% of female high school students reported engaging in PA for at least 60 minutes/day on all seven days of the week.[27] Overall, less than a quarter (23.9%) of high school students engaged in ≥60 minutes/day of PA, and a significantly lower percentage of females engaged in all PA activities assessed ($p<0.05$).[27]

In addition to PA, the YRBS collects data on dietary intake by assessing participants' consumption of fruits and vegetables, breakfast meals, sugar-sweetened beverages, sports drinks, and water.[27] According to YRBS data from 2021, 47.1% of interviewed high school students reported they consumed fruit or 100% fruit juice <1 time/day, and 45.3% had eaten vegetables <1 time/day.[27]Additionally, 75% of interviewed high school students reported they did not consume breakfast daily, and 44.2% reported drinking <3 glasses/day of plain water.[27] The DGA reports similar findings from the National Health and Nutrition Examination Survey (NHANES) 2015-2016 survey; the HEI score for children aged 2-4 years was highest (HEI=61) and decreased with increasing age to 51 in adolescents aged 14-18 years.[23] According to the DGA, the majority of children and adolescents ages 5 to 18 years under-consume the daily recommended amounts of vegetables, fruits, and dairy, and overconsume added sugars, saturated fat, and sodium.[23]

Overwhelmingly, the majority of U.S. children and adolescents are not meeting PA and dietary guidelines.[25-27] Given the established benefits of PA and diet on bone accrual, current findings indicate that a majority of children and adolescents are failing to maximize their PBM during these critical periods of skeletal growth and development.[16,24-27] Future research in children and adolescents is warranted to improve the identification and tracking

of PBM, as many youth are failing to optimize this critical factor for fracture and osteoporosis prevention.[7,16,24-27]

Osteoporosis

Osteoporosis is a systemic skeletal disease characterized by low bone mass (i.e. low bone quantity) and degraded bone microarchitecture (i.e. poor bone quality), which results in more fragile bones that are at higher risk of fracture.[28] Osteoporosis, low bone mass, and related fractures are major public health concerns that impact an estimated 53 million people in the U.S.[10] Osteoporosis and osteoporotic fractures are associated with a higher risk of mortality, in addition to increased financial burden, lower quality of life, and decreased mobility.[28] The annual number of fractures in the U.S. is projected to increase by 68%, from 1.9 million to 3.2 million from 2018 to 2040, with associated costs increasing from $57 billion in 2018 to >$95 billion in 2040.[29,30] Osteoporosis can be considered a primary or secondary disease. Primary osteoporosis results from age-related bone loss and menopause in females.[31] Whereas secondary osteoporosis results from bone-loss that is attributable to an additional clinical factor such as a disease (e.g. diabetes mellitus, chronic kidney disease, eating disorders) or treatment (e.g. glucocorticoid use).[31] Fragility fractures often precede osteoporosis diagnosis in clinical settings, and literature suggests that osteoporosis prevention and treatment is failing to address the current state of disease.[32]

Females are at greater risk for osteoporosis in comparison to males due to having shorter stature on average and thus, less aBMD in addition to low estrogen levels associated with menopause.[32] According to the International Osteoporosis Foundation (IOF), the risk of osteoporotic fracture is one in two for women aged >50 years compared to one in four for men aged >50 years.[30] aBMD is a parameter of bone quantity and is currently used to

determine fracture risk and inform the diagnosis of osteoporosis.[28] Due to aforementioned sex- and race-specific variations in bone characteristics, white females have been identified as a population at high risk of fracture compared other populations.[6,7,14] Additionally, white females have lower aBMD levels than Black females and an increased incidence of hip fractures compared to Asian females.[7]

Bone health assessment methodologies

Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DXA) is used in clinical settings to assess indicators of skeletal health and body composition. DXA is considered the "gold-standard" of bone health assessments because it is non-invasive, efficient, and low in radiation exposure compared to other methods of bone imaging.[28] DXA provides aBMD measurements which reflect the amount of mineralized tissue per specified bone area (e.g. g/cm^2). aBMD measurements are usually expressed as the number of standard deviations (SD) above or below a reference mean, which is indicated by a Z-score or T-score.[6,28,33] A Z-score compares an individual's aBMD measurement to that of a reference population of the same age, race, and sex if adequate reference data are available.[6,34] Whereas a T-score compares an individual's aBMD measurement to that of a healthy, young white female population.[34] The International Society for Clinical Densitometry (ISCD) recommends reporting aBMD as Z-scores among pediatrics, premenopausal females, and males aged <50 years and T-scores are recommended for postmenopausal females and males aged >50 years.[34] The World Health Organization (WHO) uses T-score measurements to inform the international reference standard for osteoporosis diagnostic criteria.[34] "Normal" aBMD is defined by the WHO as a T-score value ≥-1.[28] Osteopenia ("low bone density") is defined

as T-score values between -1 to -2.5.[28,34] T-score values ≤-2.5 measured at the lumbar spine (LS), total hip, or femoral neck serve as the international reference for osteoporosis diagnosis in postmenopausal females and males aged ≥50 years.[28,34] Research has characterized the inverse relationship between aBMD and fracture risk, in which low aBMD values are associated with higher risk of fracture.[35] While aBMD is a highly precise measurement and a foundational element in fracture risk prediction, limitations exist when assessing bone health and fracture risk using aBMD alone.

Bone strength and subsequently fracture risk, are partially determined by bone mass and density. The arrangement, or microarchitecture of bone also contributes to bone strength. Structural elements including cortical bone thickness and porosity and trabeculae thickness, number, and spacing influence bone strength in addition to bone mass.[7,36] aBMD from DXA does not account for characteristics of bone microarchitecture, and therefore, fails to identify individuals at risk for fracture due to poor bone quality. Fragility fractures are most common among individuals with T-score values classified in the normal (≥-1) or osteopenia aBMD (-1 to -2.5) ranges, resulting in recognition failure when using the osteoporotic reference range (≤-2.5).[31] An estimated 50% of fractures occur in individuals with T-scores greater than the osteoporotic reference range.[35] Additionally, Shevroja et al. report an overlap of up to 45% between aBMD values in individuals who fracture compared to those who do not.[28] These findings indicate relying on aBMD outcomes alone for fracture risk assessment is suboptimal and the investigation of methodologies that account for parameters of bone quality is pertinent for improving disease identification.

Trabecular bone score

Given the aforementioned limitations of DXA-derived aBMD measures, the TBS iNsight software was developed as a complimentary assessment tool for DXA. TBS iNsight software assigns a Trabecular Bone Score (TBS) to DXA LS scans by evaluating pixel gray-level variations in collected scan images to determine the arrangement of observed bone.[28] TBS is a validated index of bone microarchitecture that has been shown to correlate with 3D properties of bone microarchitecture in human cadavers.[31,37] High TBS values (>1.31) are indicative of greater numbers of trabeculae, improved bone arrangement, higher fracture resistance.[28,37-40] While low TBS values (1.23-1.31 or ≤1.23) are indicative of degraded bone microarchitecture and higher susceptibility to fracture.[28,37-40] TBS indirectly assesses bone quality, can predict fracture risk independently of aBMD, and can be used to refine current fracture-predicting tools, such as the Fracture Risk Assessment Tool (FRAX).[38] Retrospective cohort studies of women from the Manitoba Bone Density Program (n=29,407-33,352; mean age of 63-65.4 years; majority self-identifying as White) have demonstrated a consistent trend of lower fracture rates among individuals with higher TBS scores, collectively and among groups stratified according to WHO aBMD references (i.e. normal, osteopenia, osteoporosis).[33,37] Hans et al. report a modest correlation between spine aBMD and spine TBS outcomes (r=0.32).[37] However, spine TBS was found to predict fractures similarly to LS aBMD, and combining spine TBS with aBMD measurements from total hip, femoral neck, or LS scan sites resulted in significant improvement of clinical spine, hip, and any major osteoporotic fracture predictions among postmenopausal women (mean follow-up 4.7 years).[37] Additionally, Leslie et al. investigated the application of TBS among females from the Manitoba registry and

determined LS TBS was a statistically significant predictor of osteoporotic fracture risk (HR=1.18, 95% CI 1.12-1.23) when adjusting for FRAX clinical risk factors and femoral neck aBMD T-score.[33] Similar to findings from Hans et al.,[37] a complementary effect was observed when combining LS TBS and FRAX major osteoporotic fracture probability, in which a gradient in risk across TBS tertiles was observed with increasing osteoporotic fracture risk (by tertile) ($p<0.001$).[33] Collectively, these studies support the use of TBS as a complementary measurement to DXA-derived aBMD measurements and FRAX prediction equations, as including TBS in fracture risk assessment has been shown to refine fracture risk estimations.[33,37] The clinical application of TBS may be able to address the unique challenge of improving fracture risk identification among individuals with non-osteoporotic aBMD T-scores.[31,33,37]

In 2012, the Food and Drug Administration (FDA) approved the use of TBS for clinical application as a complement to DXA analyses with intentions to improve fracture risk assessment and therapeutic monitoring.[36] Over the past decade, research investigating the clinical application of TBS has significantly grown. Recently, an Expert Working Group convened by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and IOF conducted a systematic review to provide updated guidance on the clinical application of TBS in the management of osteoporosis.[31] This review included 96 articles with data on the application of TBS in men and women from over 20 countries, and sought to address the use of TBS for fracture prediction and treatment monitoring in primary and secondary osteoporosis.[31] Broadly, the ESCEO and IOF strongly recommended the application of TBS as a complement to aBMD and/or FRAX when assessing fracture risk in

postmenopausal women and men ≥50 years, and when initiating and monitoring antiosteoporosis therapy treatments (e.g. antiresorptive or anabolic agent use) in both primary and secondary osteoporosis.[31] TBS was also found to predict fracture risk independently of aBMD in individuals with secondary osteoporosis related to T2DM, chronic kidney disease (CKD), glucocorticoid treatment, and rheumatological diseases (e.g. rheumatoid arthritis).[31] Current scientific literature strongly supports the application of TBS as a complementary assessment tool in clinical practice to better capture the structural characteristics of bone, and thus, improve fracture risk prediction and antiosteoporosis treatment.[31]

Precision of DXA LS aBMD and TBS

When applying bone health assessment methodologies, such as TBS, it is critical to understand the precision of the instrument and factors that impact measurement outcomes. Assessing precision provides context to changes (decreases or increases) measurement outcomes by allowing researchers and clinical practitioners to differentiate between measurement error and true biological change. The ISCD recommends DXA facilities to conduct a precision assessment in order to determine precision error by calculating internal coefficient of variation (CV), percent coefficient of variation (%CV), and 'least significant change' (LSC) values by performing duplicate scans in >=30 people or triplicate scans in >=15 people.[34] CV values describe the relationship between standard deviation relative to a sample population's mean. Low CV values (<1) are indicative of higher precision and lower variance (dispersion) around the sample mean. CV is calculated using the root squared mean approach (RMS) supported by the ISCD guidelines, and expressed as a percentage as follows: CV (RMS-CV)=$\sqrt{\sum \frac{CV^2}{N}}$; %CV =$CV * 100\%$.[34,41] LSC

values can be calculated using CV or %CV values, in which either of these values are multiplied by 2.77 at the 95% confidence interval.[41] An LSC value serves as a reference value for discerning statistically significant changes in bone measurements (e.g. LS aBMD, TBS) from random measurement error.[41] Meaning, if a change observed over time exceeds the anticipated measurement error indicated by the LSC value, it is therefore likely biologically significant and not attributable to chance. **Figure 3** exemplifies the application of an LSC value; two patients receive DXA assessments to track their changes in bone health over a 2-year duration. Using the provided LSC values for LS aBMD (LSC=3.5%) and LS TBS (LSC=5.0%), it can be determined that neither patients experienced a statistically significant change in LS aBMD, indicating that observed changes in LS aBMD did not exceed what would be expected as a result of normal measurement error, and therefore the operator/clinician is unable to determine if the change is meaningful. However, for patient 2 (on the right side of Figure 3), the change in LS TBS (8.0%) exceeds the provided TBS LSC value (5.0%), which indicates that the observed change is greater than what would be expected due to normal measurement error, and therefore the change in LS TBS likely reflects true biological change in the skeletal tissue.

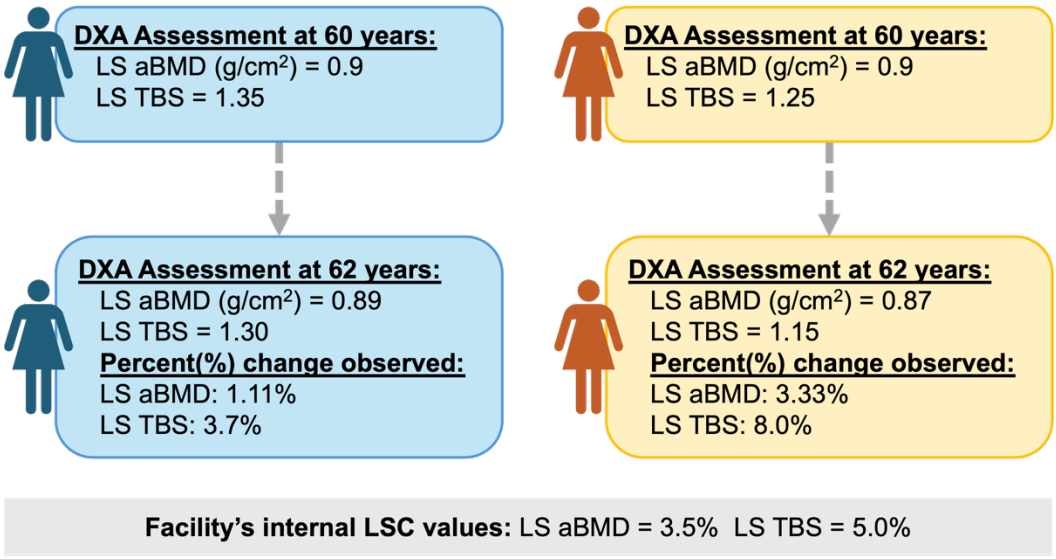

Figure 3: *Application of least significant change (LSC) values for interpreting changes in DXA LS aBMD and LS TBS outcomes in older females.*

The ESCEO and IOF found precision errors for TBS using Hologic or GE DXA systems and different TBS software versions are comparable to precision errors for aBMD in older adults.[31] Shevroja et al. report an average %CV value of 1.37% and a respective LSC value of 3.79% based on articles containing published TBS precision errors (n=11; of the total 96 studies).[31] In a prospective study of older, Caucasian females (n=90; average age 61 years), Bandirali et al. reported TBS precision values (%CV=1.9% to 2.0%; LSC=5.2% to 5.4%) that were two-fold greater than precision errors for LS aBMD (%CV=0.7% to 0.8%; LSC=2.0% to 2.1%).[42] The ISCD provides minimum acceptable precision values for LS aBMD measurements (%CV=1.9%; LSC=5.3%) obtained by individual DXA technologists.[31] Conducting a precision assessment in accordance with ISCD guidelines[34] is critical for ensuring high reproducibility of DXA-derived measures such as aBMD and TBS, and for providing context in longitudinal measurements.

Additional factors aside from operational error can impact the precision of TBS. Research has investigated the impacts of body composition and body mass index (BMI, kg/m^2) on TBS outcomes, as the presence of excessive amounts of soft tissue are thought to impact DXA scan acquisition, presenting greater challenge for differentiating texture-based variations in bone.[43] Previous versions of the TBS software (prior to version 4.0) accounted for soft tissue thickness by including BMI within the TBS algorithm; however, when applied, previous TBS versions were found to be negatively correlated with BMI or body weight.[43,44] BMI and aBMD outcomes are generally positively associated, which is supported by the aforementioned biological mechanisms of skeletal loading. Researchers speculated that the increased overlay of soft tissue was impacting TBS precision among individuals with higher BMI status.[43,44] Since, updated TBS software (version 4.0) has been released and tested in the prospective cohort OtseoLaus study.[43] The OsteoLaus study included 1,362 European, postmenopausal women (average age, 64.4; average BMI $25.9kg/m^2$) and compared the application of TBS software version 3.03 against TBS version 4.0.[43] Similar to previous findings,[44] Shevroja et al. found a negative association found between TBS and BMI when applying the version 3.03 software (r=-0.21).[43] However, when applying the updated version 4.0 software (which accounts for soft tissue thickness directly, as opposed to BMI) a positive association between TBS and BMI was found (r=0.25).[43] TBS values acquired with the updated software were significantly higher among obese women when compared to overweight or normal-weight women (p<0.01).[43] These findings indicate that recent updates to the TBS software are improving its application among individuals with greater amounts of soft tissue thickness. Given these improvements, the ESCEO and IOF encourage future investigations of TBS application in

pediatric populations, individuals with very low or high BMI values, and individuals experiencing significant weight changes.[31]

In addition to body composition and BMI, researchers have investigated if DXA scan acquisition mode impacts TBS outcomes.[42,45] Findings varied based on DXA model (Hologic vs GE), in which Bandirali et al. reported minimal differences in calculated %CV and LSC values for Hologic fast array, array, and high-definition LS scan acquisition modes.[42] Leslie et al. reports strong associations with TBS change when switching between thinner and thicker scan modes on GE DXA models.[45] These findings serve as an important reminder for DXA technologists to consider scan acquisition mode when completing initial and follow-up bone health assessments, particularly when using a GE model.

Table 1. Published LSC values for TBS.

Study Citation	Sample Population	Study design	DXA model; TBS Software Version	TBS LSC Value
Leslie et al., 2017[46]	Manitoba Density Program Registry (n=9044 postmenopausal women; mean age 61.8 ±9.8 years)	Retrospective cohort study (secondary analysis)	GE Healthcare Lunar Prodigy; TBS iNsight v2.1	0.055 (unitless)
Leslie et al., 2023[45]	Manitoba Density Program Registry (n=11,643 (93.6% female; mean age 65.3±10.0 years))	Retrospective cohort study (secondary analysis)	GE Healthcare Lunar Prodigy, iDXA; TBS iNsight v3.03	0.080 (unitless)
Kalkwarf et al., 2022[47]	Bone Mineral Density in Childhood Study (BMDCS) cohort (n=2,012; 50.7% female; 23.7% African American; ages 5 to 23 years)	Retrospective cohort study (secondary analysis)	Hologic QDR4500-A, QDR4500-W, Delphi-A; TBS iNsight v4.0 (pre-released version)	0.102 (unitless)
Sandeep et al., 2022[48]	Indian postmenopausal women (n=51; mean age 65.7±8.6 years)	Prospective cohort study (2 year follow-up)	Hologic Discovery A; TBS v3	2.2%*
Messina et al., 2019[49]	Postmenopausal Caucasian women with normal, overweight, or obese BMI status (n=95; mean age 66±10)	Cross-sectional study	Hologic QDR-Discovery W; TBS iNsight v2.1	2.52% to 4.1% (depending on BMI status)

Kang et al., 2020[50]	Adult males and females with axial spondylarthritis (axSpA) (n=240; 80% male; mean age 37±12 years)	Prospective cohort study (4 year follow-up)	GE Healthcare Lunar Prodigy; TBS iNsight v2.1	2.88%*
Kang et al., 2022[51]	Postmenopausal women (n=371; mean age 72.7 years)	Retrospective cohort study (secondary analysis)	Hologic (model(s) not identified); TBS iNsight v2.2	3.0%*
Shevroja et., 2023[31]	N/A; Systematic review of TBS literature (n=96 articles included)	Systematic review	Hologic & GE Healthcare (models not specified); multiple TBS software versions	3.8%
Guan et al., 2021[52]	Chinese adults with HIV (n=233; mean age 36.6±11.1 years)	Retrospective cohort study (secondary analysis)	GE Healthcare Lunar Prodigy Advance; TBS iNsight v2.1	3.16%*
Cosman et al., 2023[53]	Postmenopausal women (n=911; mean age 68.7±6.4 years)	Secondary analysis of RCTs	Hologic & GE Healthcare Lunar scanners (models not specified); TBS iNsight v4.0 (pre-released version)	3.75%
Kreuger, Libber, & Binkley, 2015[54]	N=335 (60.6% female; ages 22-92 years)	Retrospective cohort study (secondary analysis combining data from various studies)	GE Healthcare Prodigy, iDXA; TBS iNsight 2.1.0	3.9%; iDXA* 4.4%; Prodigy*
Breban et al., 2012[55]	Women with rheumatoid arthritis (n=185; mean age 56.0±13.5 years)	Cross-sectional study	Hologic, QDR 4500A; TBS iNsight v1.8.1.0	3.99%*
Dufour et al., 2013[56]	French Caucasian women (n=5,942; ages 45-85 years)	Retrospective cohort study (secondary analysis)	GE Healthcare Prodigy, Lunar; TBS iNsight v1.8	4.1%
Bilezikian et al., 2018[57]	Postmenopausal women (n=138; mean age 66.2 years)	Retrospective cohort study (secondary analysis)	Not identified	4.2%
Hans et al, 2011[58]	N/A; Human cadaver vertebrae (n=30)	Exploratory study	GE Healthcare Prodigy; TBS iNsight software not identified	4.51%
Bandirali et al., 2015[42]	Caucasian postmenopausal women (n=90; mean age 61-62 ± 9-10 years)	Cross-sectional study	Hologic QDR-Discovery A; TBS iNsight v02)	5.2% to 5.4% (depending on scan mode)
Shepherd et al., 2006[59]	N/A	Review conducted for 2005 ISCD Official Positions	Reviewed studies included Hologic, GE Healthcare, & Norland Medical densitometers	5.3% (not specific to TBS; LS LSC reference guideline)

McClung et al., 2017[34,60]	Postmenopausal women (n=285; mean age 72.5±5.0 years)	Retrospective cohort study (secondary analysis)	GE Healthcare Prodigy, iDXA, Hologic QDR 4500, Discovery, Horizon; TBS iNsight v1.9	5.82%

*TBS LSC value calculated from %CV value(s) reported in cited article.

Investigating in vivo TBS precision in young adult females

The findings from the current project will allow for the refinement of previously published LSC values for TBS (maximum LSC value reported is 5.4%).[42] Refining %CV and LSC values for TBS will allow future researchers to interpret changes in skeletal mass and microarchitecture with greater precision, as the current maximum LSC value of 5.4% is highly conservative in comparison to reported aBMD LSC values ranging from 2.0-2.1%.[42] Reducing the accepted TBS LSC value of 5.4% would reflect minimized variation between scans (resulting from patient positioning and differences in scan analyses) and allow for more precise measures of biological changes in skeletal health status. Increased precision in bone health assessment tools such as TBS would result in more effective screening, prevention, and treatment methods for fractures and osteoporosis. With improved osteoporosis prevention, medical costs associated with fractures and low bone mass could be reduced, and more importantly, individuals at risk for osteoporosis could be identified earlier in life and treated to minimize age-related bone loss and reduce fracture risk. Investigating the application of TBS in young adult females is warranted, as assessing both bone quantity and bone quality during the acquisition and maintenance of PBM in a population at increased risk, could allow for the optimization and tracking of this protective factor in osteoporosis prevention.

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CHAPTER 3

IN VIVO PRECISION OF DUAL ENERGY X-RAY ABSORPTIOMETRY LUMBAR SPINE TRABECULAR BONE SCORE IN YOUNG ADULT FEMALES

Introduction

Osteoporosis is a skeletal disease characterized by low bone mass and poor bone microarchitecture, which results in more fragile bones that are at higher risk of fracture.[1] Osteoporosis typically occurs due to age-related bone loss and is most prevalent among older adults.[2-4] Low bone mass and osteoporosis are major public health concerns that impact an estimated 54 million people in the U.S.[2,5] In 2018, annual costs associated with osteoporotic fractures were estimated to be $57 billion and predicted to increase to >$95 billion by 2040.[6] Annually, an estimated 1.5 million individuals experience an osteoporotic fracture.[4] Osteoporotic fractures are associated with a higher risk of mortality, increased financial burden, decreased mobility and independence, and lower quality of life.[1] Fragility fractures often precede osteoporosis diagnosis, indicating that current prevention and treatment methods are failing to address the current state of disease.[7]

Despite its prevalence among older adults, osteoporosis has origins in childhood and adolescence, which are critical periods of bone accrual.[2,4,8,9] Previous research has demonstrated the "tracking" of bone mass and density during childhood and adolescence, indicating that the establishment of healthy bones early in life can reduce osteoporosis and fracture risk in older adulthood.[10,11] PBM, defined as the maximum amount of bone an individual will accrue, is attained during the transitional period from late-adolescence to

young adulthood and is a major determinant of osteoporosis and fracture risk later in life.[8,9] Simulated models have shown that a 10% increase in PBM could delay the onset of osteoporosis by 13 years for many individuals.[8] Essentially, optimizing PBM attainment during the first three decades of life could mitigate future age-related bone loss.[8] PBM is influenced by genetic (e.g. sex, age, race, ethnicity) and lifestyle factors (e.g. physical activity, diet, sleep), which contributes to its wide variation. This presents challenge in identifying PBM attainment at an individual-level with current bone health assessment practices and methodologies.[8]

Dual energy X-ray absorptiometry (DXA) is the current *gold standard* for bone health assessment in osteoporosis prevention and treatment.[1] aBMD a DXA-derived measure of bone mass, is currently used to determine fracture risk and diagnose osteoporosis. Previous research has established BMD as a highly precise measure that is inversely related to fracture risk.[12] However, BMD does not account for bone microarchitecture and therefore fails to identify fracture risk in people with poor bone quality. Fifty percent (50%) of fragility fractures occur in individuals with BMD T-scores above the osteoporotic range (>-2.5), posing a unique clinical challenge for effectively assessing an individual's risk of fracture and osteoporosis.[12]

Trabecular Bone Score (TBS) is new measure of spine bone quality that can assess trabecular bone using pixel gray-level variations in LS DXA scan images to determine the arrangement of bone.[1] The LS is rich in trabecular (spongy) bone, which is metabolically active and sensitive to skeletal loading from mechanical forces (e.g. physical activity). Trabecular bone is composed honeycomb-like structures called trabeculae, which form connections to disperse imposed loads; generally, greater connectivity within trabecular

bone indicates increased-resistance to fracture. Although it has historically been presumed that PBM is maintained until ageing-related bone loss commences in older age, recent data from a longitudinal study in healthy adolescents showed that consolidation of trabecular bone might commence as early as the late teenage years.[13] Since the LS is comprised of mostly trabecular bone, this particular skeletal region might be particularly vulnerable to subtle losses in bone during the adolescent-to-young adult transition.

When applying bone health assessment methodologies, it is critical to assess the precision of employed techniques in order to determine expected measurement error from true biological change. The International Society for Clinical Densitometry (ISCD) provides published guidelines for conducting a precision assessment study to encourage facilities to calculate internal precision values to help inform interpretation of longitudinal measurements. The objective of this study is to determine the in vivo precision of TBS, a new measure of LS bone quality, in a sample of young adult females ages 18 to 30 years. Using published guidelines from the ISCD, we will 1) compare the in vivo precision of TBS and LS aBMD and 2) determine subject characteristics that associate with the precision of TBS, including BMI, abdominal soft tissue thickness, total body fat, among others. We hypothesize that TBS will be highly precise and comparable to that of LS aBMD in a sample of young adult females, and that demographic characteristics such as BMI, soft tissue thickness, and total body fat will not significantly impact TBS and LS aBMD precision.

The proposed research is significant because the precision of TBS has not yet been determined in young adult females, a population at increased risk for osteoporosis later in life. The expected outcomes of this study will enhance the current application and

understanding of TBS outcomes in patient populations at risk of osteoporosis and fracture. Our findings will advance current bone health assessment methods by generating an internal LSC value for the University of Georgia's (UGA) Nutrition and Skeletal Health Laboratory and contributing to previously published precision error (%CV) and LSC values for TBS.

Subjects and methods

We enrolled a sample of 19 healthy young adult females ages 18 to 30 years to participate in this cross-sectional study. The desired sample size was based on published precision assessment guidelines from the International Society for Clinical Densitometry (ISCD), which recommends bone densitometry facilities to determine internal in vivo precision error by measuring at least 15 patients (from the intended patient population) three times with repositioning on the densitometer prior to each scan.[1] Subjects were screened prior to participating in the study using an online survey, and those who were eligible were invited to enroll by a trained research professional. Inclusion criteria included: female sex, being between the ages of 18 and 30 years, and having a self-reported body mass index (BMI; kg/m^2) within the 'healthy weight' or 'overweight' range (i.e., BMI between 18.5 and 29.9 kg/m^2 for individuals ≥30 years of age and BMI-for-age percentile between the 5^{th} and 95th percentile[2,3]). Exclusion criteria were male sex, being unable to read/understand English, having a significant (>10% of initial body weight) weight change in the past six months, prior diagnosis of osteoporosis, prior hip or spine fracture, any fracture within the last year, medical or surgical implant that contraindicates bone density evaluation, and being pregnant. A urine pregnancy test was administered at the beginning

of each study visit to confirm eligibility for a dual-energy X-ray absorptiometry (DXA) exam.

Subjects participated in one laboratory visit completed at the University of Georgia (UGA) Nutrition and Skeletal Health Laboratory (**Figure 4**). Prior to participating in the study, all subjects provided written informed consent. All study protocols and procedures were approved by the UGA Institutional Review Board for Human Subjects.

Anthropometric measurements

Standing height and weight were measured using a wall-mounted stadiometer and digital scale, respectively. All anthropometric measurements were performed in triplicate and averaged together by a single trained researcher. BMI was calculated and BMI-for-age percentile was calculated for subjects <20 years of age.[2,3]

Health history, physical activity, and diet

Health history and demographics questionnaires were completed for each study subject to obtain data on healthy history/status, race, ethnicity, menstrual health, disease and fracture history, and medication and/or contraceptive use (e.g. oral contraceptives, intrauterine device). Physical activity (PA) for the week (7 days) prior to a subject's study visit was measured using the long form of the International Physical Activity Questionnaire (IPAQ).[4,5] The long form IPAQ assesses five domains related to PA including leisure time PA, domestic and yard activities, work-related PA, transport-related PA, and time spent sitting.[4,5] Information regarding PA intensity and duration are also captured by the long form IPAQ questionnaire.[4,5] Diet was assessed by trained research staff via 24-hour dietary recalls using the Nutrition Data System for Research (NDSR) software.[6] A multiple pass

method was used to collect self-reported dietary intake for the day prior to a subject's study visit. Total energy intake, as well as other nutrients known to influence bone (e.g. protein (g), calcium (mg), and vitamin D (mcg)) were collected.[6] All questionnaires were completed at the study visit and administered by a single researcher who received training from a Registered Dietitian Nutritionist.

Dual-energy X-ray absorptiometry

In accordance with ISCD precision assessment guidelines, each subject completed a series of DXA scans in triplicate at a single study visit. A series of DXA scans included a total body, LS (L1-L4 vertebrae), non-dominant hip, and non-dominant forearm scan (**Figure 4**). Between each series of DXA scans, subjects were removed from the densitometer for a rest period of one to five minutes and then were subsequently repositioned upon the scanner for the next series of scans. All scans were performed using a Hologic Horizon densitometer (Hologic, Inc.). Calibration of the densitometer was performed prior to each study visit using a spine phantom provided by Hologic, Inc. A single trained researcher performed and analyzed all DXA scans using APEX software version 2.1 and TBS iNsight version 3.1.2. LS aBMD and TBS Z-scores were computed using published reference ranges from NHANES III. For subjects <20 years of age (n=4), an age of 20 year was manually assigned for TBS analyses and TBS Z-score calculations.

Precision error calculations

Once data collection was completed, batch analyses of the collected DXA scans were conducted. LS aBMD and TBS analysis results were used to CV, %CV, and LSC values in order to determine the precision of LS aBMD and TBS. CV was calculated using

the root squared mean approach (RMS) supported by the ISCD guidelines,[1,7] and expressed as a percentage as follows: CV (RMS-CV)=$\sqrt{\sum \frac{CV^2}{N}}$; $\%CV*100\%$. LSC values can be calculated using CV or %CV values, in which either of these values are multiplied by 2.77 at the 95% confidence interval.[7] An LSC value serves as a reference value for discerning statistically significant changes in bone measurements (e.g. LS aBMD, TBS) from random measurement error.[7] Differences in bone measurements must meet or exceed a calculated LSC to be considered statistically significant.[7] The ISCD encourages facilities to conduct a precision study to determine internal LSC value for use when assessing changes of aBMD and TBS overtime.[1] The ISCD has published an online Microsoft Excel-based calculator for the calculation of RMS standard deviation (RMS-SD), CV, %CV, and LSC values, which was used in this study to calculate the aforementioned precision assessment parameters.[1]

Statistical analyses

CV values for LS aBMD and TBS were calculated for each subject using the ISCD calculator and LS aBMD and TBS results from each subject's triplicate of LS DXA scans. Paired t-tests were performed to compare the precision error (%CV) for TBS, LS aBMD, and total body less head (TB) aBMD to determine whether the precision of these measures differs. The relationship between %CV for TBS and %CV for LS aBMD was assessed via Pearson's correlation and visualized using a scatter plot. Associations between precision values (%CV) for LS aBMD and TBS and subject characteristics (e.g. BMI, abdominal soft tissue thickness, total body fat) were assessed via Pearson correlation and visualized using scatter plots. Additional analyses included descriptive statistics reported as mean and

standard deviation and percentage of sample population for binary variables (e.g. sex, race, ethnicity). All analyses were performed using STATA version 15.

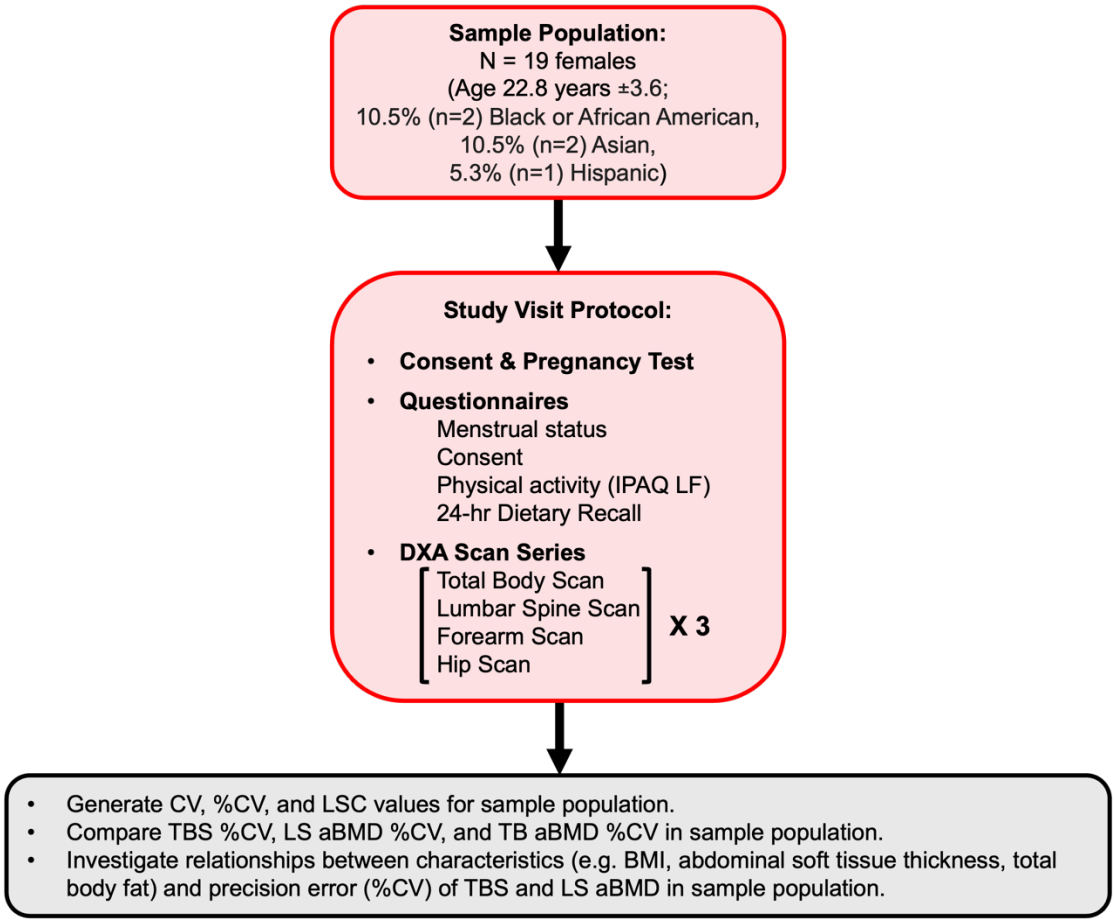


Figure 4: Study Design. *This is a cross-sectional study in which young adult females completed one study visit which included questionnaires, a 24-hour dietary recall, and DXA scans to determine and compare LS TBS and LS aBMD outcomes.*

Results

Descriptive Statistics

Descriptive statistics are presented in **Table 2**. This study sample included young adult females ages 18-30 years (n=19), with an average age of 22.8 (SD=3.6) years. 73.68% (n=14) participants self-identified as White, 10.53% (n=2) participants self-identified as African American or Black, 10.53% (n=2) participants self-identified as Asian, and 5.26% (n=1) participants self-identified as Hispanic. Average BMI of the sample was 23.9 (SD=3.3) kg/m^2.

Compared to the 2020-2025 DGA, the average reported caloric intake for the sample population fell within the estimated daily calorie needs for "moderately active" or "active" females ages 18 to 30 years (2,000-2,400 kcals).[14] The sample population's average reported macronutrient profile exceeded DGA recommendations.[14] However, when considering the high amounts of PA this sample population reported engaging in, caloric and macronutrient intake may be less than optimal for maintaining energy balance. All participants met (n=7) or exceeded (n=12) PA guidelines of completing ≥150 minutes of moderate-to-vigorous PA per week.[14] Additionally, average reported intake of fruit and vegetable servings exceeded U.S. Department of Agriculture (USDA) MyPlate guidelines (1.5-2.5 cups/day and 2.5-3 cups/day, respectively).[14] However, average reported calcium, vitamin D, fiber, and servings of dairy intake were less than DGA recommendations for females ages 18 to 30 years (1,000-1,300 mg/day, 600 IU/day, 25-28 g/day, 3 cups/day, respectively).[14]

Table 2. Participant characteristics.

Subject characteristic	**Mean±SD**
Age, years	22.8±3.6
Self-reported race, n(%)	
White (%)	14 (73.68%)
Black or African American	2 (10.53%)
Asian	2 (10.53%)
Hispanic	1 (5.26%)
Height, cm	163.6±4.9
Weight, kg	64.1±10.3
BMI, kg/m^2	23.9±3.3
Total fat (%)	28.7±5.5
Soft tissue thickness, cm	6.5±0.6
Lumbar spine aBMD, g/cm^2	1.05±0.12
Lumbar spine aBMD, Z-score	0.07±1.13
Lumbar spine trabecular bone score	1.49±0.06
Lumbar spine trabecular bone score, Z-score	0.22±0.59
Total calories, kcals	2127.1±705.9
Macronutrients, g	
Carbohydrate	257.0±87.0
Protein	76.8±38.8
Fat	87.7±32.0
Calcium, mg	783.2±336.5
Vitamin D, mcg	2.27±2.2
Fiber, g	21.9±12.1
Fruit, servings	2.2±2.1
Vegetables, servings	3.9±2.9
Dairy, servings	1.1±0.9
Total physical activity, MET-min/week	4387.1±3696.9

Total vigorous physical activity, MET-min/week	1450.1±1719.1
Total moderate physical activity, MET-min/week	1106.3±1063.2
Total walking, MET-min/week	1830.6±1910.0
Physical activity categories, n(%)	
Low	0 (0%)
Moderate	7 (36.8%)
High	12 (63.2%)

Fruit servings are defined per 2000 DGA as one medium apple, orange, or pear, ½ cup of chopped, cooked, or canned fruit, ¼ cup of dried fruit, or 4 fl oz of 100% fruit juices; vegetable servings are defined per 2000 DGA as 1 cup of raw leafy vegetables or ½ cup of other cooked or raw vegetables; Dairy servings are defined per 2000 DGA as approximately equivalent to the amount of calcium in 1 cup of milk or yogurt, 1 ½ oz of natural cheese, and 2 oz of processed cheese (FDA serving sizes used when DGA do not apply); MET-min/week = number of minutes of physical activity (vigorous, moderate, or walking) x MET value (8.0, 4.0, or 3.3, respectively).

In vivo precision of DXA bone outcomes

%CV and LSC values for lumbar spine (LS) aBMD, LS TBS, and TB aBMD are presented in **Table 3**. The LSC values for TBS, LS aBMD, and TB aBMD ranged from 2.11% to 2.46% (**Table 3**).

Table 3. Precision error values calculated using ISCD calculator.

Bone measure	**RMS SD**	**%CV (%)**	**LSC[a]**	**LSC (%)**
TBS	0.0132 (unitless)	0.89	0.0366 (unitless)	2.46
LS aBMD	0.0088 (g/cm^2)	0.88	0.0244 (g/cm^2)	2.44
TB aBMD	0.0075 (g/cm^2)	0.76	0.0209 (g/cm^2)	2.11

RMS SD = root mean square standard deviation; %CV= percent coefficient of variation; LSC[a] calculated from RMS SDx2.77; LSC (%) calculated as %CVx2.77.

All DXA bone measures were highly precise, and precision error (%CV) for these outcomes did not differ significantly from one another (**Figure 5**). The average %CV for TBS, LS aBMD, and TB aBMD are 0.82 ±0.35, 0.72% ±0.52, and 0.69% ±0.33, respectively. Average %CV for TBS did not significantly differ from average %CV for LS aBMD (P=0.5096) or average %CV for TB aBMD (P=0.2776). Similarly, average %CV for LS aBMD did not significantly differ from average %CV for TB aBMD (P=0.8192). Additionally, the association between %CV for TBS and %CV for LS aBMD (assessed via Pearson's correlation) was not statistically significant (r=0.034, P=0.892).

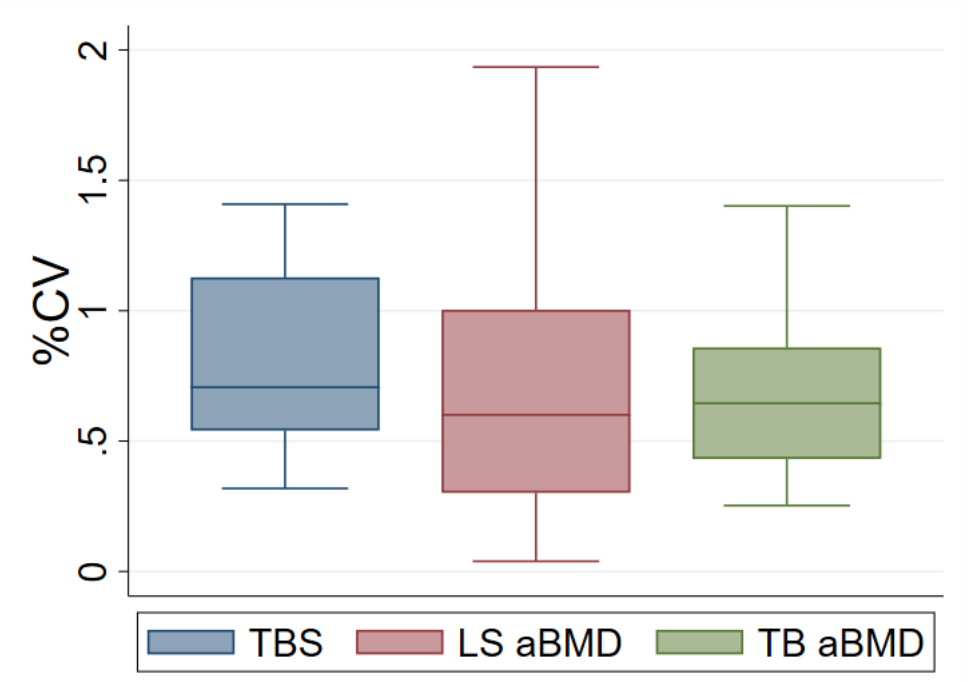
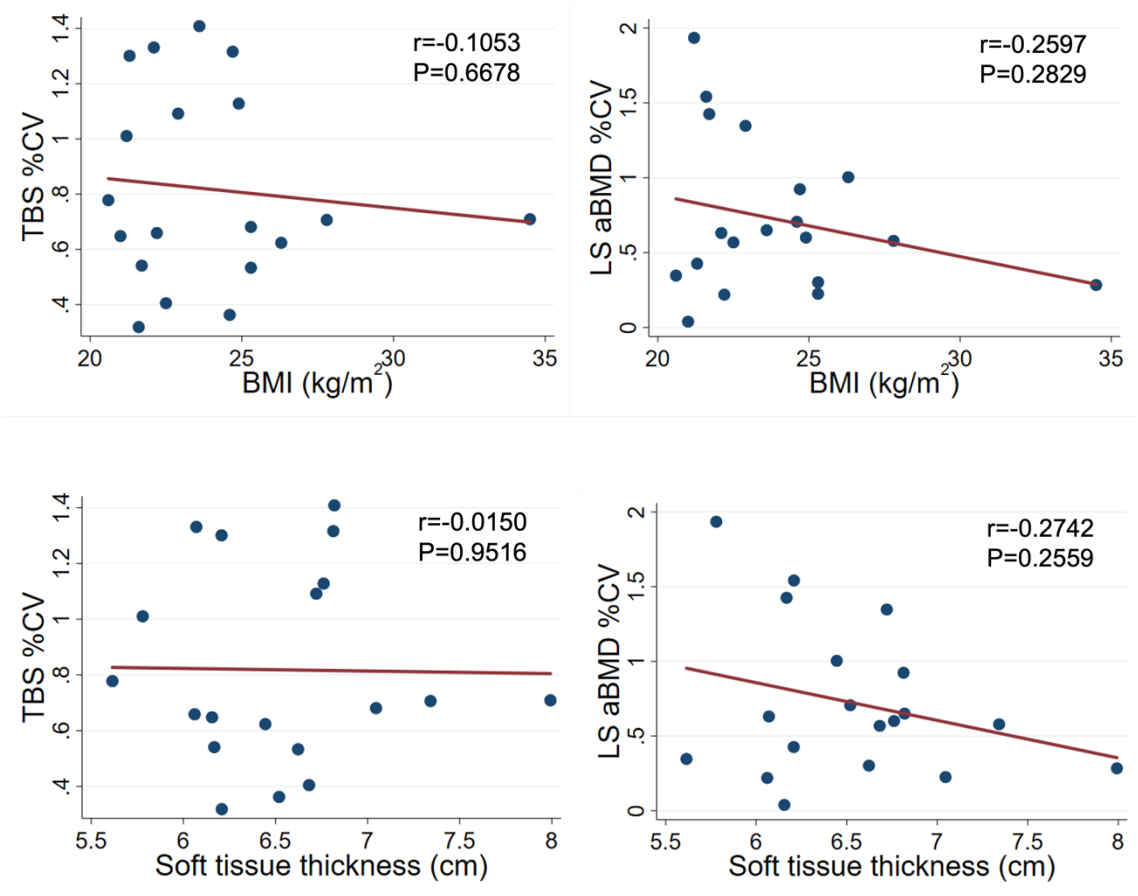


Figure 5. *Paired t-test results for average %CV TBS, LS aBMD, and TB aBMD; all P values >0.05.*

Associations between body size and in vivo precision for DXA bone outcomes

Associations between measures of body size and %CV for TBS and LS aBMD are presented in **Figure 6**. TBS %CV and LS aBMD %CV values did not correlate with BMI (P=0.6678; P=0.2829, respectively). Similarly, TBS %CV and LS aBMD %CV values did not correlate with soft tissue thickness (P=0.9516; P=0.2559, respectively), nor total fat (%) (P=0.7044; P=0.7021, respectively). A sensitivity analysis which excluded a participant with a BMI >29.9 kg/m^2 was conducted and all associations were maintained upon the exclusion of this participant (results not reported).



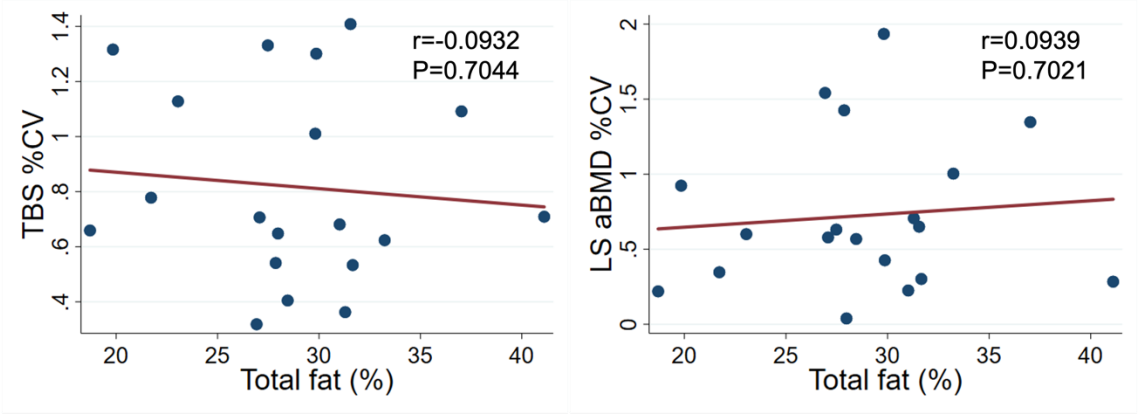


Figure 6. *Associations between average %CV values for TBS or LS aBMD and BMI, soft tissue thickness, or total fat.*

Discussion

Precision error (%CV) and LSC values generated for TBS and LS aBMD in this study were highly precise and did not differ from one another. Additionally, precision error for TBS and LS aBMD did not differ from that of TB aBMD, which was included in statistical analyses to compare precision error measurements from the LS to those from the whole (or "total") body. These findings indicate that TBS and LS aBMD are both highly precise measures in healthy young adult females ages 18 to 30 years.

The ISCD guidelines recommend DXA facilities to conduct internal precision assessments to generate precision error values including %CV and LSC. Establishing internal LSC values for aBMD and TBS allows clinicians and researchers the ability to discern true biological change in bone from measurement error, particularly when interpreting longitudinal DXA outcomes. Given that TBS is an emerging measure of bone health for clinical use, it is critical to determine the precision of this bone health assessment tool across the lifespan. Many studies have investigated the application and precision of TBS in older adult populations, including postmenopausal women due to their increased

risk of osteoporosis and fracture. Despite the prevalence of osteoporosis among older adults, determining the precision of TBS in young adult populations is warranted, as PBM, a key determinant of osteoporosis risk, is attained within the second to third decades of life.

To our knowledge, this is the first study to investigate the precision of TBS in a cohort of healthy young adult females ages 18 to 30 years. This is an important sample of the population to study as PBM is attained between the second and third decades of life, and recent longitudinal studies have shown that trabecular bone consolidation begins as early as adolescence and young adulthood. Standard aBMD measures alone may not fully capture these nuanced changes in trabecular bone; therefore, investigating the application of TBS is warranted in this sample population. The main finding from this study was that TBS and LS aBMD were both highly precise measures in young adult females, and did not significantly differ from each other. Calculated TBS %CV and LSC values generated using the ISCD calculator were similar to those of LS aBMD and TB aBMD, indicating that TBS was as precise as LS aBMD and TB aBMD measures in our sample population. A recent study investigated the precision of TBS in children and adolescents (n=2,012; ages 6 to 16 years), and reported an average TBS %CV value for this population of 2.87%, which was 2- to 3-times greater than that reported for LS aBMD (%CV=0.85%).[1] Additionally, TBS precision error was found to decrease with age and approached values similar to those found in older adult populations among adolescents 14 to 16 years old (%CV=1.21 to 1.9%).[1] Findings from the present study do not reflect a significant difference between internal TBS and LS aBMD precision error (%CV) in a sample of healthy females ages 18 to 30 years. Additionally, the precision error of TBS (%CV=0.89%) from the present study was found to be approximately threefold less than the average TBS %CV reported for

children and adolescents. [1] Whereas LS aBMD precision error from the present study (%CV=0.88) was found to be similar to that reported in children and adolescents (%CV=0.85). [1] Collectively, these preliminary findings in children, adolescents, and young adult females suggest that TBS precision improves with age, with %CV values approaching those found in older adult populations and minimum acceptable precision recommendations from the ISCD by late adolescence and early adulthood.

When compared to recent studies, TBS %CV and LSC values generated in the present study 1) align with those published within the past five years,[2-6] 2) are less than precision error averages reported by the ESCEO and IOF, [7] and 3) comply with current ISCD guidelines. [8] Previously reported LSC values for TBS range from 3.9% to 5.82% in older adults.[9-13,15] More recent studies (2019-present) have reported LSC values ranging from 2.2% to 3.75% among sample populations of postmenopausal women.[2-4,16] The recent refinement of published TBS precision error values may be reflective of technological (e.g. densitometer upgrades, TBS iNsight software updates) and protocol advancements in clinical and research bone health assessment practices (e.g. increased ISCD guideline compliance). Based on studies to date, the ESCEO and IOF report an average precision error (%CV) of 1.4% for TBS with a corresponding LSC of 3.8%.[7] TBS precision error and LSC values from the present study fall below that reported average (%CV=0.89%, LSC=2.46%). Additionally, the aforementioned minimum acceptable precision error values provided by the ISCD are %CV ≤1.9% and LSC ≤5.3% for the LS scan site (for an individual technologist).[8] The reported precision error and LSC values for both TBS and LS aBMD comply with these ISCD guidelines. Comparing precision error values reported

in the present study to these references further exemplifies the precision of TBS in our sample of young adult females.

Multiple factors can impact the precision of DXA outcomes, including densitometer model, scan mode, technologist/operator skill with respect to both scan acquisition and analysis, and subject characteristics such as age and body size.[1,17,18] Subject characteristics, including BMI, abdominal soft tissue thickness, and total fat (%) were not associated with internal TBS or LS aBMD precision error (%CV) in the present study. This suggests that BMI, abdominal soft tissue thickness, and total fat (%) did not impact the precision of TBS or LS aBMD in our sample of young adult females. It's important to note, however, that our study did not include individuals with obesity, and therefore cannot be applied to these individuals. Our findings are consistent with those from previous studies investigating the relationships between TBS precision error and BMI or soft tissue thickness. A phantom study investigating the impacts of soft tissue overlay (simulated by fresh pork rind) on TBS and LS aBMD precision, found that TBS precision was lower than that of LS aBMD, but was not significantly affected by ≤6cm of simulated soft tissue thickness and was less influenced by soft tissue overlay compared to aBMD.[19] To expand on these findings, Messina et al. conducted an in vivo precision assessment study in accordance with ISCD guidelines to investigate TBS precision among postmenopausal Caucasian women (n=95; mean age 66±10 years) with normal weight (<25kg/m^2), overweight (25-29.9kg/m^2), and class I obesity (≥30kg/m^2) BMI status.[4] When comparing TBS and LS aBMD precision error across different BMI and waist circumference (WC; ≤88cm, >88cm) groups, no statistically significant differences were found for TBS or LS aBMD precision error between groups, with %CV values ranging from 1.21%-1.53% and

0.91%-1.48%, respectively.[4] While no significant differences in precision error were observed between BMI and WC groups, TBS precision was found to be slightly lower than that of LS aBMD in the normal weight (<25kg/m^2, P<0.001) and overweight BMI groups (25-29.9kg/m^2, P=0.01) in addition to both WC groups (≤88cm, P<0.001; >88cm, P<0.001).[4] These findings contrast a previous study from Knapp et al., which found that LS aBMD and femoral neck aBMD precision error (%CV) both increased significantly in overweight and obese females compared to those with normal weight BMI (n=102; ages 30 to 65 years).[18] Furthermore, total body fat (%) was found to increase LS aBMD precision error (%CV) when comparing females with 30%-≥45% total fat to females with <30% total fat (P≤0.05).[18] TBS precision error values were not reported by Knapp et al..[18] Further investigation is needed in young adults with overweight and obesity to better understand the impacts of BMI, abdominal soft tissue thickness, and total body fat on TBS precision.

Key strengths of this study include the application of novel bone methodologies (i.e. TBS) in populations that have yet to be investigated and compliance with ISCD precision assessment guidelines. Furthermore, despite the relatively small (n=19) sample size, 26.3% (n=5) participants self-identified as non-white which reflects greater racial/ethnic diversity than many previous studies investigating TBS. A limitation to this study is that all precision error and LSC values were generated from scans collected and analyzed by a single trained researcher. The ISCD recommends averaging precision error calculations for all technologists in a given DXA facility when conducting a precision assessment study.[8] Failure to include precision error calculations for additional technologists in this study may contribute to the comparably low TBS %CV and LSC

values that were generated. While low precision error is preferred, %CV and LSC values should be reflective of an entire facility's technologist staff in order to determine the true amount of measurement error observed as opposed to generating precision error values using a single technologist. All DXA scans were completed with a Hologic Horizon densitometer (Hologic, Inc.), which presents limitations when applying our calculated %CV and LSC values to DXA measurements obtained with GE Healthcare densitometers. Lastly, this study included a small sample (n=19) of healthy young adult females. While this sample population meets ISCD guidelines for a precision assessment study, the external validity of our findings may be limited by sample size and the exclusion of individuals of male sex or with self-reported BMI values >29.9kg/m^2.

In conclusion, TBS was found to be highly precise in young adult females (ages 18 to 30 years), with precision error and LSC values comparable to those for LS aBMD. The results from this study, namely the LSC values, can be used by the UGA Nutrition and Skeletal Health lab and others to monitor longitudinal changes in bone in females during the critical years surrounding PBM. Future research investigating TBS precision among children, adolescents, and young adults of minority populations, varying weight status, and conditions known to impact bone development is warranted, as improving bone health assessment methodologies could be critical in optimizing the achievement of PBM and preventing osteoporosis.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

The objective of this thesis was to 1) determine the precision of TBS, a new measure of bone quality of the LS in young adult females, 2) generate internal precision error (%CV) and LSC values for the UGA Nutrition and Skeletal Health Laboratory, and 3) contribute to the current %CV and LSC values for TBS in the scientific literature. The study presented in Chapter 3 was conducted with the primary aim of determining and comparing the in vivo precision of TBS and LS aBMD in young adult females ages 18 to 30 years. Our findings indicate that TBS is a highly precise measure in healthy young adult females and which precision error comparable to LS aBMD and TB aBMD. Specifically, average %CV values for TBS (0.8185% ±0.3515), LS aBMD (0.7238% ±0.5155), and TB aBMD (0.6902% ±0.3320) did not differ from one another (all $P > 0.05$). Additionally, associations between %CV values for TBS and LS aBMD and body size parameters including BMI, soft tissue thickness, and total body fat were characterized as a secondary aim of the study. No significant associations were found between precision error (%CV) of TBS or LS aBMD and these body size parameters (all $P > 0.05$). These results indicate that TBS is a highly precise measure, with precision error (%CV) comparable to that of LS aBMD, in healthy young adult females. Future investigation of TBS precision and validation is warranted among children, adolescents, and young adults of minority populations including non-white race/ethnicity, various

diseases and conditions impacting skeletal development, and various body sizes to further understand the precision of TBS.

The findings from this study address a gap in the current state of scientific literature, as LSC values in young adult females have not been reported to our knowledge. Determining the precision error (%CV) and LSC values for populations across the lifespan is critical for interpreting changes TBS and LS aBMD outcomes from longitudinal DXA assessments, particularly when administering and monitoring the effects of anti-osteoporotic medications. As aforementioned, future research is pertinent to support and advance the clinical application of TBS during the years surrounding PBM attainment.

APPENDICES I

In vivo precision of dual energy X-ray absorptiometry lumbar spine trabecular bone score in young adult females

CONSENT FORM

DEMOGRAPHICS QUESTIONNAIRRE

HEALTH HISTORY QUESTIONNAIRE

MENOPAUSE QUESTIONNAIRRE

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRRE (IPAQ)

APPENDIX A

CONSENT FORM

UNIVERSITY OF GEORGIA
CONSENT FORM

Researcher's Statement: You are being asked to take part in a research study. Before you decide if you may participate in this study, it is important that you understand why the research is being done and what it will involve. This form is designed to give you the information about the study so you can decide whether you wish to be in the study or not. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all of your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent." A copy of this form will be given to you.

If you are interested in participating in the study, please read the additional information on the following pages, and feel free to ask questions at any point.

Principal Investigator: *Dr. Joseph M. Kindler, PhD, CTR*
Nutritional Sciences
kindlerj@uga.edu
717-798-0776

Study Coordinator: *Ms. Staci Belcher, MS, RDN, LDN*
Nutritional Sciences
stacibelcher@uga.edu
706-542-7466

- The purpose of this study is to measure the precision of a bone density scanner in our research lab.
- You are being asked to participate in this research study because you are female and between the ages of 18 and 30 or are 50 years of age or older.
- Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled.
- This research study involves one appointment at the Nutrition and Skeletal Health Lab (1-1.5 hours), which is located in room 275 Dawson Hall on the main UGA campus (305 Sanford Drive, Athens GA)
- During the visit, you will be asked to complete anthropometrics (height, weight), health-related questionnaires, a dietary recall, and bone health assessment measures.
- Risks include exposure to a small amount of radiation and possible psychological discomfort while answering questionnaires.

If you are interested in participating in the study, please read the additional information on the following pages, and feel free to ask questions at any point.

Study Procedures and Time Commitment: As a participant in this study, you will be asked to complete the following procedures at one study visit. The study visit will be completed at the Nutrition and Skeletal Health Laboratory (Dawson Hall, Office Suite 279), and will last approximately 1-1.5 hours.

Questionnaires (10 minutes): All questionnaires will take place in a private office space with one research team member. All questionnaires will be administered by a trained researcher, your responses will be stored in an encrypted research database, called "RedCap."

- Menopause questionnaire: Brief questionnaire involving questions related to menstrual health
- Health history questionnaire: Brief questionnaire involving overall health status, bone fractures/injuries, medication use, and chronic health conditions.
- Demographics Questionnaire: This questionnaire includes questions regarding your race/ethnicity and sex.
- Physical activity questionnaire: This questionnaire includes questions regarding your typical activity levels and specific activities you commonly participate in.

Body size (5 minutes): Height (using a wall mounted stadiometer), weight (using an electronic scale), arm length (using a ruler), and leg length (using a sliding caliper) will be assessed. A trained researcher will perform all measurements. An additional study team member will be present for all measurements.

Dual-energy X-ray absorptiometry (DXA) (30 minutes): The DXA machine, pictured on the right, is a specific X-ray machine used for bone density assessment. The DXA machine is located in a private room in the Nutrition and Skeletal Health Lab. Several DXA scans will be completed by a trained research team member to assess bone density and body composition. The research team member has undergone radiation safety training and device-specific training. DXA scans of your whole body, lumbar spine (lower back), forearm, and hip will be completed. A total of 13 scans will be completed. The duration of each scan ranges from a couple of seconds to a couple of minutes. Scans will be performed in a laying or seated position. Clothing with metal is prohibited as it interferes with quality of your scans. You will be provided with light clothing and a private space to change if your clothing contains metal.

24-hour diet recall (25 minutes): The dietary recall is collected through an interview with a trained study team member. This interview includes questions regarding what foods/beverages and how much of each food/beverage you consumed in the previous 24 hours. The 24-hour recall is interview administered and responses are inputted directly to Nutrient Data Systems for Research software. The dietary recall will be completed in a private quiet room in the Nutrition and Skeletal Health Laboratory.

Potential Risks and Discomforts: There are some potential risks and/or discomforts associated with the procedures outlined above.

Radiation exposure: You will be exposed to a small amount of radiation during the DXA scans. The total radiation from the DXA scans result in about 52 µSV of radiation. For comparison, a standard dental X-ray totals about 20-40 µSVs, a standard chest X-ray totals about 50-150 µSVs, and a flight from the east coast to

the west coast of the United States totals about 50 μSVs. In the event that a scan is unusable due to poor quality, additional scans might be performed but limited to two repeat scans.

Being a part of this study while pregnant may expose the unborn child to a yet undiscovered risk. Therefore, all participants who consent to the study who have not gone through menopause will take a pregnancy test. If pregnant, your participation will end, however, you will still receive the research incentive.

Embarrassment/discomfort from questionnaires: You may experience some psychological discomfort from the disclosure of information relating to health history information or demographics. You may skip any question that may be distressing. If undue discomfort or stress occurs, you have the right to discontinue participation in the study at any time.

Benefits of Participation: There are no potential benefits of participating in this study.

Incentive: If you complete the study visit, you will receive a $20 check mailed to your address 2-6 weeks after the completion of the study. If you elect to withdraw from the study during the study visit, or if you are withdrawn from the study due to a positive pregnancy test, you will receive the full payment of $20. In order to process the payment for your participation, the researcher(s) need to collect your name and mailing address on a separate payment form. This completed form will be sent to the Department of Nutritional Sciences' business office and then to the UGA Business Office. The researcher has been informed that these offices will keep your information private, but may have to release your name and the amount of compensation paid to you to the IRS, if you earn over $600 from UGA in one year. The researcher connected will store this information in a secured location.

Goodwill and scientific advancement: The results generated from this study will help assess the precision of the DXA. This study will help in the methodological development of future studies aimed at reducing risk for osteoporosis and fracture. Additionally, this research will help determine most appropriate clinical approaches for identifying people with osteoporosis.

Privacy and Confidentiality: Though some individually-identifiable information will be collected from you for contact purposes, all data collected as part of the study procedures will be coded using a subject ID, which consists of letters and numbers. The key to the above mentioned code will be kept in a password protected computer file. Only the researcher and members of this research team will have access to identifiable data. The project's research records may be reviewed by departments at UGA responsible for regulatory and research oversight. The key to the code matching your name with your ID number will be destroyed following a ten-year retention period. Researchers will not release identifiable results of the study to anyone other than individuals working on the project without your written consent unless required by law.

Voluntary Consent: Your participation in this study is voluntary. You can refuse to participate or stop taking part at any time without giving any reason, and without penalty or loss of benefits to which you are otherwise entitled. If you decide to withdraw from the study, the information that was previously collected will be kept as part of the study and may continue to be analyzed, unless you make a written request to remove, return, or

destroy the information. If you are a student, your academic/lab standing or grades will not be impacted in any way by participating in this study, or choosing to withdraw from this study. You will not receive individual research results; no research data will be disclosed to study participants.

Use of Data in Future Studies: If you consent to your data being used in future studies, use of this data will be limited to ten years. All of your data will be deidentified. These data include: bone and body composition measurements and/or questionnaires. You will not be informed of the details of any scientific research studies that might be conducted using your private information; this research would relate to factors affecting bone strength and growth and you may not have chosen to consent to some of those specific research studies. We may collaborate with other academic institutions in these future studies and share the deidentified data with these institutions. It is possible that your data will be combined with data from other similar studies that used identical procedures.

If you are injured by this research: The researchers will exercise all reasonable care to protect you from harm during your participation in this study. In the event of an injury as an immediate and direct result of participation, the researchers' sole responsibility is to arrange for transportation to an appropriate facility if additional care is needed. If you think that you have suffered a research-related injury, you should seek immediate medical attention and then contact Dr. Joseph Kindler right away at 717-798-0776. In the event that you suffer a research-related injury, the medical expenses will be your responsibility or that of your third-party payer, although you are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research.

If you have questions: The main researcher conducting this study is Dr. Joseph Kindler, an Assistant Professor at UGA. Please ask any questions you have now. If you have questions later, you may contact Dr. Kindler at kindlerj@uga.edu or 717-798-0776. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at UGA at 706-542-3199 or irb@uga.edu.

Continue to next page…

Questions to be answered by the study participant:

Use of Research Data for Future Study: I give the researchers of this study permission to use my data in future studies.

Yes: _____ No: _____

Research subject's consent to participate in research

To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form, and have had all of your questions answered.

_____	_____	
Name of Researcher	Signature	Date
_____	_____	
Name of Participant	Signature	Date

Continue to next page…

Future Contact: I give the researchers of this study permission to contact me regarding participation in future research studies.

Yes: _____ No: _____

**if yes, please provide the following information*

Name:

_____

Address:

_____

Telephone: _____

Email (work/school): _____

Email (person): _____

Please sign both copies, keep one and return one to the researcher.

APPENDIX B

DEMOGRAPHICS QUESTIONNAIRRE

DXA Precision Study

Demographics Questionnaire

1. What is your date of birth?

   Month: _____

   Day: _____

   Year: _____

2. What do you consider your race? You can indicate more than one.
   - American Indian or Alaskan Native
   - Native Hawaiian or Pacific Islander
   - Asian
   - White
   - Black or African American

3. Do you consider yourself to be Hispanic or Latino?
   - Yes
   - No

APPENDIX C

HEALTH HISTORY QUESTIONNAIRRE

DXA Precision Study

Health History Questionnaire

1) Are you currently taking any medications?

☐ Yes
☐ No

If 'yes,' please list the medications, dose, and reason for use

Medication	Dose	Reason for Use

2) Are you currently taking any dietary/herbal supplements?

☐ Yes
☐ No

If 'yes,' please list the dietary/herbal supplements, dose, and reason for use

Supplement	Dose	Reason for Use

3) Have you ever broken a bone before?

☐ Yes
☐ No

If 'yes,' please provide information regarding each time that you have broken a bone:

	Which specific bone did you break?	At what age did the fracture occur?	How did the fracture occur?
Broken bone 1			
Broken bone 2			
Broken bone 3			

4) Have you ever been diagnosed by a doctor with any of the following health conditions?

- ☐ Any cancer
- ☐ Type 1 diabetes
- ☐ Type 2 diabetes
- ☐ Hypertension
- ☐ Arthritis
- ☐ Alzheimer's
- ☐ Other: _____
- ☐ None

5) Are you currently under the care of a doctor for any ongoing medical problems?

- ☐ Yes
- ☐ No

If yes,
specify:_____

6) Have you ever taken any form of birth control?

- ☐ Yes
- ☐ No

7) Are you currently using any form of birth control?

- ☐ Yes
- ☐ No

If 'yes' to either of the two previous questions, please provide information regarding each type of birth control you have ever used

Mode (patch, implant, pill, injection, etc.)	Name of birth control	Dose	Duration of use	Currently taking? (yes or no)

APPENDIX D

MENOPAUSE QUESTIONNAIRRE

DXA Precision Study

Menopause Questionnaire

1) What age did you have your first menstrual period? _____

2) What was the approximate date of your last menstrual period (month and year)? _____

3) How would you define your menstrual cycle? Circle one:
   a. Regular
   b. Irregular
   c. Absent (I am in menopause)

**"Menopause" is defined as not having a menstrual period in the past 1 year*

4) If you answered "Irregular" or "Absent" for Question 3, at what age did your menstrual cycle first become irregular? _____

5) If you answered "Absent" for Question 3, at what age did you consider yourself "post-menopausal?" _____

6) If you answered "Absent" for Question 3, are you currently taking any medication for your menopause?
   □ Yes → please specify: _____
   □ No

APPENDIX E

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRRE (IPAQ)

IPAQ Long Last 7 Days Self Admin

Record ID _____

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport. Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

◯ Yes
◯ No > Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work?

Think about only those physical activities that you did for at least 10 minutes at a time.

◯ 1 day per week
◯ 2 days per week
◯ 3 days per week
◯ 4 days per week
◯ 5 days per week
◯ 6 days per week
◯ 7 days per week
◯ No vigorous job-related physical activity > Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

hours per day _____

minutes per day _____

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No moderate job-related physical activity > Skip to question 6
5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?	
minutes per day	_____
hours per day	_____
6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No job-related walking > Skip to PART 2: TRANSPORTATION
7. How much time did you usually spend on one of those days walking as part of your work?	
hours per day	_____
minutes per day	_____

PART 2: TRANSPORTATION PHYSICAL ACTIVITY These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car or tram?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No traveling in a motor vehicle > Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?	
hours per day	_____
minutes per day	_____

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?

- ◯ 1 day per week
- ◯ 2 days per week
- ◯ 3 days per week
- ◯ 4 days per week
- ◯ 5 days per week
- ◯ 6 days per week
- ◯ 7 days per week
- ◯ No bicycling from place to place > Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?

hours per day _____

minutes per day _____

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?

- ◯ 1 day per week
- ◯ 2 days per week
- ◯ 3 days per week
- ◯ 4 days per week
- ◯ 5 days per week
- ◯ 6 days per week
- ◯ 7 days per week
- ◯ No walking from place to place > Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR

13. How much time did you usually spend on one of those days walking from place to place?

hours per day _____

minutes per day _____

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?

- ◯ 1 day per week
- ◯ 2 days per week
- ◯ 3 days per week
- ◯ 4 days per week
- ◯ 5 days per week
- ◯ 6 days per week
- ◯ 7 days per week
- ◯ No vigorous activity in garden or yard > Skip to question 16

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

hours per day	_____
minutes per day	_____
16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No moderate activity in garden or yard > Skip to question 18
17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?	
hours per day	_____
minutes per day	_____
18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No moderate activity inside home > Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY
19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?	
hours per day	_____
minutes per day	_____
PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.	
20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No walking in leisure time > Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?	
hours per day	_____
minutes per day	_____
22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No vigorous activity in leisure time > Skip to question 24
23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?	
hours per day	_____
minutes per day	_____
24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?	◯ 1 day per week ◯ 2 days per week ◯ 3 days per week ◯ 4 days per week ◯ 5 days per week ◯ 6 days per week ◯ 7 days per week ◯ No moderate activity in leisure time > Skip to PART 5: TIME SPENT SITTING
25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?	
hours per day	_____
minutes per day	_____

PART 5: TIME SPENT SITTING The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?	
hours per day	_____

minutes per day

_____

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?

hours per day

_____

minutes per day

_____

This is the end of the questionnaire, thank you for participating.

This physical activity questionnaire is publically available, it is open access, and no permissions are required to use

it. So we encourage any researchers to use it where it will be an appropriate measure of physical activity, particularly in larg
https://sites.google.com/site/theipaq/