

UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE

RACIAL/ETHNIC DIFFERENCES IN BONE STATUS, MUSCLE FUNCTION, AND
FAT MASS, IN YOUNG AND MIDDLE-AGED PREMENOPAUSAL WOMEN
BELONGING TO CAUCASIAN, EAST-ASIAN, AND SOUTH-ASIAN BACKGROUNDS

A DISSERTATION
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
Degree of
DOCTOR OF PHILOSOPHY

By
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Norman, Oklahoma
2019

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A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ACKNOWLEDGMENTS

Firstly, I would like to express my sincere gratitude and heartfelt thanks to my research supervisor, Dr. Michael G. Bemben, for his invaluable guidance, patience, and motivation. I thank him for providing me with this opportunity and experience, his expertise, endless support, and in believing in me throughout my Ph.D. program.

I am extremely grateful to my committee members, Drs. Debra Bemben, Rebecca Larson, Jason Campbell, and Michael Crowson, for their invaluable input, insightful comments, and encouragement for the successful completion of this project. My special thanks to Dr. Debra Bemben for her advice and support in the design and execution of this project. I thank my fellow lab mates, especially Ryan, Eduardo, and Aaron, for their help, the stimulating discussions and all the fun we had.

The completion of this project would not have been possible without all those people who participated in this study. I sincerely appreciate their contributions and gratefully acknowledge them.

I thank my family, parents, siblings, and brother-in-law, for their constant support and encouragement. Last, but not the least, thank you, Aditya, for your unconditional support and motivation, and for making this journey so special and amazing for me.

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ABSTRACT

Introduction: Racial/ethnic differences in bone mineral density (BMD) result in increased susceptibility of some ethnic groups to fragility fractures in comparison to others. Although Asians have a lower or equivalent BMD in comparison to Caucasians, they have a significantly lower non-vertebral fracture risk. However, most of these studies are focused on East-Asians, especially Chinese, or do not take Asian sub-groups into consideration. Osteoporosis incidence and fractures rates vary in the Asian sub-groups. Unlike East-Asians, osteoporotic fractures occur 10-20 years earlier in South-Asian women when compared to Caucasian women.

Studies determining BMD and its determinants in South-Asians using standardized equipment such as Dual Energy X-Ray Absorptiometry (DXA) are scarce and not well defined. Therefore, in order to reduce the physical and economic burden of osteoporosis, it is important to assess BMD and its determinants considering East- and South-Asians as independent racial/ethnic groups.

Purpose: The purpose of the current study was to determine group differences and relationships between bone mineral density, bone free lean body mass and muscle strength, and fat mass, in premenopausal women aged 18-45 years belonging to three different racial/ethnic groups: Caucasians, South-Asians (SA), and East-Asians (EA). For each ethnicity, the given age range (18-45 years) was divided into two sub-groups: 18-30 years (young), and >30 to 45 years (middle-aged), to allow comparison between women who are accruing bone mass vs. those who have achieved their peak bone mass. Serum concentrations of vitamin D were assessed to further understand the age and ethnicity related differences between these tissues.

Methodology: This was a non-randomized cross-sectional study consisting of 107 participants. Based on their race/ethnicity the participants were categorized into one of the three independent

racial/ethnic groups: Caucasian (Cau; n= 46); East-Asian (EA; n= 29); and South-Asian (SA; n= 27). On the basis of age, each ethnicity was further sub-divided into two groups: 18-30 years (young (n= 65; Cau= 24, EA= 24, SA= 17)); and >30-45 years (middle-aged (n=38; Cau= 22, EA= 10, SA= 10)). The first visit included completion of documentation such as the consent form, HIPAA, ethnicity identification form, calcium and vitamin D food intake questionnaires, health status questionnaire, menstrual history questionnaire, physical activity readiness questionnaire (PAR-Q), bone specific physical activity questionnaire (BPAQ), international physical activity questionnaire (IPAQ), and sun exposure questionnaire. This was followed by resting blood pressure measurements and familiarization of the participants with muscle strength tests. The second visit consisted of a blood draw to analyze serum concentrations of vitamin D (25(OH)D) and follicle stimulating hormone, for women >40 years. During the third visit measurements of bone mineral density (areal (a) and volumetric (v) BMD), and body composition (bone mineral content (BMC), bone free lean body mass (BFLBM), whole-body fat mass) were performed using Dual-Energy X-Ray Absorptiometry and peripheral Quantitative Computed Tomography. Following this, upper and lower body muscle strength was measured through the handgrip test, jump test, and 1RM leg press test.

Data Analyses: Analyses were done using SPSS (SPSS Inc., Chicago, IL, version 24.0). Data were presented as mean \pm SE. A two-way analysis of covariance was conducted using height, weight, and duration of stay in the United States as covariates, to assess the main effects of age and ethnicity and Ethnicity X Age interactions. This was followed by a Bonferroni post-hoc test to determine group differences between the three ethnic groups. Zero-order Pearson correlation coefficients were used to assess relationships between the dependent variables. Chi-square analyses were conducted between ethnicity, and other categorical variables to determine

association and sampling distribution. Finally, multiple linear regression analysis was conducted to examine the association between dependent and independent variables.

Results: Areal BMD at the left femoral neck was higher in Caucasians compared to East-Asian women, and higher in younger Caucasian women compared to young South-Asians. At 38% of the tibia, total vBMD was higher in Caucasians than East- and South-Asian women, and SSI was higher in Caucasians compared to South-Asian women. Endosteal circumference was higher in East-Asian than in Caucasian women, whereas, polar moment of inertia was higher in East-Asians compared to South-Asian women. At 66% of the tibia, total BMC was higher in East-Asian compared to South-Asian women, whereas, cortical BMC was higher in both Caucasian and East-Asian women compared to South-Asians. Total body fat percentage and fat mass were significantly greater in South-Asian women than East-Asians and Caucasians. Moreover, Android/Gynoid ratio was significantly higher in East-Asians compared to Caucasian women. Serum vitamin D levels were higher in Caucasians compared to both East- and South-Asians, whereas sun exposure scores were higher for East-Asians compared to South-Asians. In addition to this, significant positive correlations were noted between age, height, weight, age of menarche, body composition and muscle strength variables, and calcium and vitamin D intakes, and areal and volumetric BMD and bone strength parameters for the three ethnic groups. Moreover, BFLBM, handgrip strength, and physical activity scores were significant predictors of lumbar spine and femoral neck aBMD and bone strength parameters for Caucasians and East-Asians, whereas, fat mass, BFLBM, and physical activity predicted these parameters in South-Asians.

Conclusions: Our results demonstrate that femoral neck aBMD is lower in East-Asians compared to Caucasians. Moreover, in the young groups, South-Asian women have a lower

femoral neck aBMD compared to young Caucasians. South-Asians also have a lower vBMD, BMC, and bone strength parameters than East-Asians and Caucasians. This, along with their decreased serum vitamin D levels, sun exposure scores, higher fat mass, and lower leg muscle strength can potentially help to explain the early incidence of osteoporotic fractures in this population. The results of this study can be used for creating awareness among the at-risk ethnicities regarding the importance of adequate physical activity and dietary practices in enhancing bone density. Moreover, the inferences derived from this study can be used to design exercise programs that are ethnicity specific and more effective in preventing osteoporotic fractures.

CHAPTER I

INTRODUCTION

Bone is a specialized dynamic tissue that maintains structure and mechanical integrity of the body, provides protection to the vital organs, and supports hematopoiesis and mineral homeostasis (Morgan, Barnes, & Einhorn, 2013). The skeleton is metabolically active and undergoes remodeling throughout life by maintaining a balance between breakdown of the old bone, *resorption*, and buildup of the new bone, *formation* (Rucci, 2008). Bone remodeling is tightly regulated by the activity of three types of cells: osteoblasts, osteoclasts, and osteocytes, to keep the bone mass constant. The bone remodeling cycle can take 3-6 months to complete and includes removal of the old bone by osteoclasts, followed by secretion of the new bone matrix by osteoblasts. Some of the osteoblasts become entrapped within the bone matrix and differentiate into osteocytes (Kini & Nandeesh, 2012). The osteocytes respond to mechanical stresses acting on the bone and through their highly interconnected canalicular network signal to the osteoblasts and osteoclasts on the bone surface and regulate their activity, thereby fulfilling their role as the '*mechanosensors*' (Prideaux, Findlay, & Atkins, 2016).

In normal healthy individuals, bone formation is coupled with bone resorption, thus maintaining homeostasis (Morgan et al., 2013). Any disturbance to this coupling mechanism results in greater amount of bone resorption in comparison to formation, thereby reducing bone mineral density and increased skeletal fragility. This condition is known as '*Osteoporosis*' and is characterized by reduction of both bone quantity and quality, resulting in fractures with minimal trauma most commonly at the hip, spine, and forearm (Burge et al., 2007; Rosen & Bouxsein, 2006). Bone mineral density (BMD) is a strong predictor of osteoporotic fractures and each standard deviation decrease in bone density increases the risk of fracture by 1.5 to 2.6-

fold (Siris et al., 2004). For diagnostic purposes, BMD values are expressed in terms of T-scores, with a T-score of -2.5 or below defining osteoporosis, and between -1 to -2.5 indicating osteopenia, a clinical precursor to osteoporosis (Dougherty & Al-Marzouk, 2001). The current gold standard for measuring BMD is Dual Energy X-Ray Absorptiometry (DXA), which represents BMD as a two-dimensional measurement (g/cm^2), *areal* BMD (Cong & Walker, 2014). DXA measures areal BMD (aBMD) and bone mineral content (BMC) at the total body and at specific skeletal sites.

Osteoporosis is a potentially debilitating disease that deteriorates bone tissue resulting in approximately 2 million fractures in the United States (U.S.) each year, incurring a financial burden of 22.4 billion dollars (Burge et al., 2007). It is reported that the lifetime risk of osteoporosis related morbidity in women is greater than the combined risk for breast, endometrial and ovarian cancers (Pothiwala, Evans, & Chapman-Novakofski, 2006). The majority of these osteoporotic fractures occur in women (71%) (Burge et al., 2007). In addition to gender, race/ethnicity is a critical factor determining the incidence and prevalence of osteoporosis as it is associated with genetics, that account for 60-80% variation in adult bone mass, and other lifestyle factors such as physical activity and nutrition (Heaney et al., 2000; Rosen & Bouxsein, 2006).

It is well documented that African-American women have a higher BMD than Caucasians, both at the appendicular and axial skeleton, owing to their lower fragility fracture rates at all skeletal sites (Conradie, Conradie, Kidd, & Hough, 2014). The National Health and Nutrition Examination Survey (NHANES)-III reported that the prevalence of osteoporosis is lower in Black women juxtaposed to non-Hispanic Whites (Wallace, Ballard, Holiday, & Wells, 2005). The higher BMD in African-American women is attributed to either their higher peak

bone mass, or to an attenuated rate of bone loss during adulthood, or both (Conradie et al., 2014).

Another largest growing population in the U.S. are the Asians. Asians have a similar or lower axial and non-axial BMD than Caucasians, but their non-axial fracture rates are significantly lower in comparison to Caucasian women (Boutroy et al., 2014; Cong & Walker, 2014). This contradicts the apparent link between lower BMD and higher fracture risk. Ethnic groups vary by geographical region and culture in Asia. The Asian ethnicity is comprised of East-Asian, South-Asian, North-Asian, Southeast-Asian, West-Asian, and Central-Asian sub-groups. Prevalence of osteoporosis and fracture incidence varies within these Asian sub-groups, however, most studies examining BMD and its determinants in Asians are focused on East-Asians or do not take Asian sub-groups into consideration (Cong & Walker, 2014). East-Asian is a term representing ethnic groups native to East-Asia, which includes mainland China, Hong Kong, Macao, Taiwan, Japan, South Korea, North Korea, and often Mongolia and Vietnam (Rashidvash, 2015).

Unlike East-Asians, osteoporotic fractures occur 10-20 years earlier in South-Asian women when compared to Caucasian women (Makker, Mishra, Singh, Tripathi, & Singh, 2008). South-Asians (SA) is a term representing natives from India, Bangladesh, Bhutan, Maldives, Nepal, Pakistan and Sri Lanka (Rashidvash, 2015). Studies determining BMD and its determinants in South-Asians using standardized equipment such as Dual Energy X-Ray Absorptiometry (DXA) and peripheral Quantitative Computed Tomography (pQCT) are scarce and mostly based on surrogate measures such as Singh's index, calcaneal index, visual assessment, quantitative ultrasound, and digital X-ray radiogrammetry. Therefore, to reduce the

physical and economic burden of osteoporosis, it is critical to assess BMD and its determinants considering East- and South-Asians as independent racial/ethnic groups.

Along with race/ethnicity, BMD is determined by muscle mass and strength, and fat mass, since these tissues are placed in close proximity to the bone and interact with the bone at both mechanical and biochemical levels. Skeletal muscle-derived mechanical loading is a major factor regulating bone density, along with genetics, nutrition, hormones, and growth factors. Mechanical loads above the routine threshold deform or strain the bones, thereby adapting them to become stronger. However, it is reported that muscle mass along with its strength and quality, begins declining towards the end of the third decade of life, with poor nutrition, physical inactivity, chronic disease, and drug therapy, accelerating this loss (Ormsbee et al., 2014). This progressive loss of muscle mass and strength is termed as '*Sarcopenia*' - Greek for '*poverty of flesh*'. Sarcopenia and osteoporosis are often present together in the same patient and represent the *chicken and egg situation* where it is difficult to deduce which one precedes the other. The mechanical perspective suggests that muscle loss leads to bone loss, however, low bone mass patients are not always sarcopenic (Bonewald, 2019).

In addition to the bone-muscle unit, the bone-fat unit is also considered crucial for bone health as increased mechanical load due to higher body weight, attributable to excessive fat mass, is conventionally linked to a higher BMD. However, it has been reported that this positive relationship between excess body weight/fat mass and BMD becomes detrimental following the adjustment for its mechanical loading effects (Yoo et al., 2012). Also, low body weight does not necessarily mean low body fat. A higher fat mass has been documented in Asians who otherwise weighed less in comparison to their White counterparts and were reported to have a lower BMD (Gallagher et al., 2000). Thus, unlike the bone-muscle unit, the bone-fat unit is complex, and not primarily dependent on the mechanical loading effects of excess body weight/fat mass.

Loss of muscle mass and strength and gain of fat mass are a consequence of decreased physical activity levels and result in late-life functional impairment (Ormsbee et al., 2014). Physical activity provides mechanical loads beneficial to bone health by maintaining or increasing muscle mass/strength. However, some ethnic groups lack awareness regarding the benefits of physical activity in enhancing cardio-metabolic fitness and maintaining bone and muscle strength, apart from merely reducing or maintaining body weight. A cross-sectional study reported that significantly higher percentage of Whites were engaged in high and moderate level physical activities as compared to Blacks and Hispanics (Vásquez, Shaw, Gensburg, Okorodudu, & Corsino, 2013). Similarly, it has been reported that South-Asian women have a lower rate and a low desire to participate in physical activities, which may be a potential contributor to the higher obesity rates in this population. This may be due to differences in ideologies where higher body weight is considered aesthetic and linked to prosperity, differences in socio-economic status, and cultural and religious restrictions (Ranasinghe, Ranasinghe, Jayawardena, & Misra, 2013).

Higher physical activity levels are also linked to increased serum vitamin D levels in both men and women (Kaur et al., 2019). Earlier classified as a nutrient, vitamin D is a potent steroid hormone. $1,25(OH)_2D$ is the primary hormonal form of vitamin D in which it performs its biological actions. It regulates bone metabolism via direct and indirect routes and is important for mineralization of the bone tissue. Although natural food sources of vitamin D are limited, it can be synthesized endogenously in the skin by the ultra-violet rays of the sun (Feldman, Krishnan, & Swami, 2013). However, variations in zenith angle, latitude, season, skin pigmentation, use of sunscreen, use of protective clothing, and amount of time spent outdoors, limit the amount of radiation reaching the earth's surface or being absorbed by the body, thus

leading to restrictions in cutaneous synthesis of vitamin D. This results in vitamin D deficiency/insufficiency even in individuals living in areas where there is ample sunlight most months of the year (Morgan et al., 2013; van Driel & van Leeuwen, 2017). Serum vitamin D level (25(OH)D) ≥ 30 ng/mL is classified as sufficient vitamin D, while levels between 21-29ng/mL and below 20ng/mL are classified as insufficient and deficient respectively (Holick, 2009; Lee, Gadi, Spertus, Tang, & O'Keefe, 2011).

Bone mineral density is reported to be greater or similar in high muscle/low fat and high muscle/high fat body types, however, an increase in fat mass ($\geq 32\%$ of body weight) without a concurrent increase in muscle mass, results in decreased BMD. Ilich et al. (2014), proposed the term 'Osteo-Sarcopenic Obesity' (OSO) representing the simultaneous derangement of these three tissues (Figure 1). This odd combination involves loss of bone (osteoporosis) and muscle (sarcopenia) in the presence of or as a result of excessive fat mass (obesity).

Initially regarded as a condition restricted to the elderly population, OSO is now suggested to have an earlier, sub-clinical form that initiates during young adulthood and silently progresses to express itself later in life, *early-onset progressive osteosarcopenic obesity* (Stefanaki, Peppas, Boschiero, & Chrousos, 2016). Therefore, taking into consideration the codependency of the bone-muscle-fat unit, it is vital to assess these three tissues concurrently, keeping bone at the forefront.

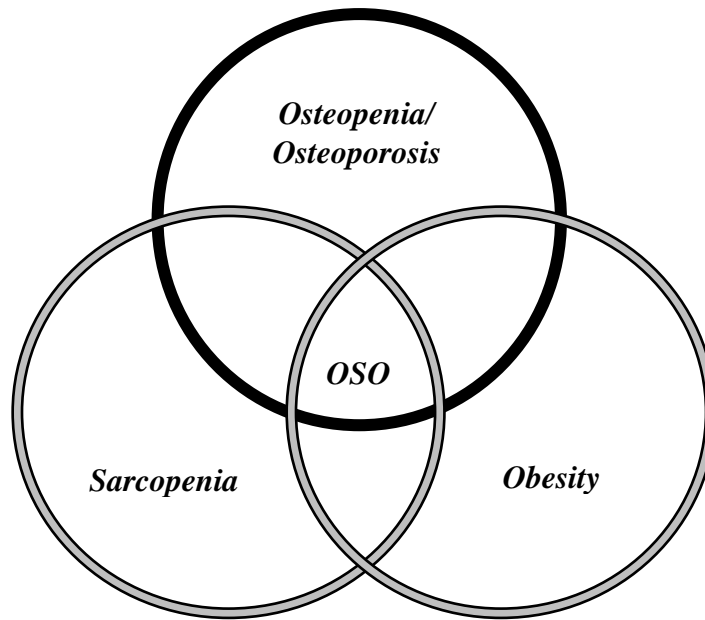


Figure 1: Diagrammatic representation of the derangement between bone, muscle, and fat resulting in OSO (Adapted from Ilich et al., 2014, with modifications).

Purpose

The primary purpose of this study was to determine group differences and relationships between bone mineral density, bone free lean body mass and muscle strength, and fat mass, in premenopausal women aged 18-45 years belonging to three different racial/ethnic groups:

Caucasians, South-Asians (SA), East-Asians (EA). For each ethnicity, the given age range (18-45 years) was divided into two sub-groups: 18-30 years (young), and >30 to 45 years (middle-aged), to allow comparison between women who are accruing bone mass vs. those who have achieved their peak bone mass.

The central hypothesis for this study was that South-Asian women will have reduced bone mineral density in comparison to Caucasian and East-Asian women. South-Asians will also have a lower lean mass and muscle strength, and higher fat mass, leading to increased

fracture risk compared to East-Asian and Caucasian women. These differences will be related to lifestyle factors such as physical activity, and calcium and vitamin D intake (Figure 2). The pilot data for this study indicated that young South-Asian women have a higher fat mass and lower bone free lean body mass (BFLBM) and muscle strength than Caucasians and East-Asians. We hypothesized that these changes will intensify with age, explaining the early bone loss and fracture incidence reported in this population. Moreover, the current study also examined serum concentration of vitamin D, to further understand the underlying biochemical pathways responsible for these differences.

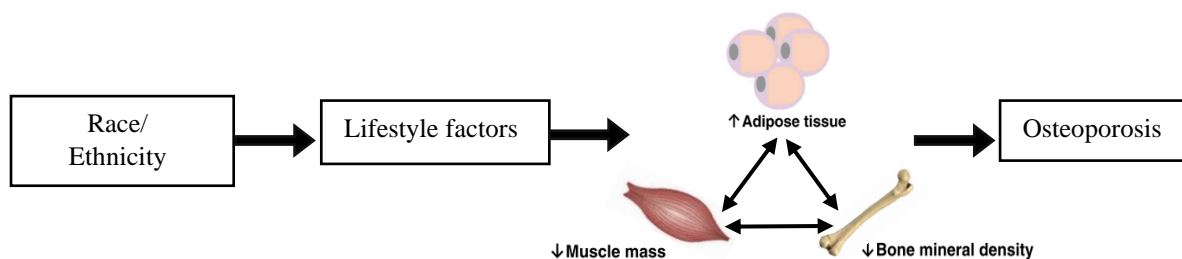


Figure 2: Theoretical model for the central hypothesis of the study depicting the influence of race/ethnicity on bone, muscle and fat tissues mediated via lifestyle factors

Research Questions

1. Is there a significant difference in bone status, areal and volumetric BMD, in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?
2. Is there a significant difference in body composition parameters, BFLBM, and muscle strength, and fat mass, in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

3. Is there a significant interaction between age groups (young vs. middle-aged) and race/ethnicity (Caucasians, EA, SA), for bone status (areal and volumetric BMD) and body composition parameters (BFLBM and strength, fat mass)?

Hypotheses

1. Areal and volumetric BMD will be highest for Caucasians, followed by EA, and least for SA. These ethnic differences in bone density will persist across the two age groups. However, in each ethnic group as well as across the entire sample, younger women (18-30 years) will tend to have a higher areal and volumetric BMD in comparison to middle-aged women (>30-45 years).
2. Caucasians will have the highest, and SA will have the lowest BFLBM and muscle strength. Whole-body (WB) fat mass will be highest for SA, followed by EA, and least for Caucasians. These differences in BFLBM, muscle strength, and fat mass, will persist across the two age groups. However, in each ethnic group as well as for the entire sample, younger women (18-30 years) will have a higher BFLBM and muscle strength, and lower WB fat mass, compared to middle-aged women (>30-45 years).
3. There will be no significant interaction between age and race/ethnicity, as both the age groups will demonstrate similar patterns for bone density and body composition for the three racial/ethnic groups, Caucasians, EA, and SA.

Sub-questions

1. Is there a significant difference in circulating vitamin D levels in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

2. Is there a significant difference in physical activity (PA) levels in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

Sub-hypotheses

1. Serum vitamin D levels will be highest for Caucasians, followed by EA and least for SA. These differences will be related to lighter skin color, higher dietary and supplemental intake of vitamin D, and greater sun exposure in Caucasians compared to SA. These differences will persist across the two age groups. However, in each ethnic group as well as for the entire sample, younger women (18-30 years) will have higher vitamin D levels compared to middle-aged women (>30-45 years).
2. Caucasians will have the highest PA levels, followed by EA, and least in SA. These differences will persist across the two age groups. However, in each ethnic group as well as for the entire sample, younger women (18-30 years) will have higher PA levels compared to middle-aged women (>30-45 years).

Significance of the study

As per prior literature, peak bone mass is attained by the end of the third decade of life. Thus, examining bone health in a young age group (18-30 years in the current study) becomes vital as this provides an opportunity to optimize peak bone mass through adjustments in lifestyle factors like nutrition and physical activity and decrease the risk for current and future fractures. For premenopausal women aged >30-45 years, peak bone mass has already been achieved, and an accelerated bone loss due to menopause is impending. Thus, assessment of BMD and its predictors can help to augment efforts to maintain bone mass through dietary interventions and

structured exercise programs, so that a clinical diagnosis of osteopenia/osteoporosis can at least be delayed.

Variations in education, socio-economic status, and access to healthcare, results in disparities in health-related disorders, with differences being more pronounced in those belonging to minorities. A study by Shakil et al. (2010), assessed the awareness of osteoporosis and its risk factors among South-Asian women residing in the U.S. They reported that 77% of the participants were not aware that osteoporosis can cause bone fractures and that calcium deficiency and physical inactivity are its major risk factors.

The Asian American Health Initiative was established in 2005 to eliminate the health disparities between Asians and non-Asians living in the United States. This society conducts outreach events providing osteoporosis screening and creating awareness regarding its preventative strategies in Maryland, U.S. However, not all states in the U.S. have such programs available and these programs focus only on women above 50 years of age when bone loss due to menopause cannot be reversed. This indicates that although osteoporosis awareness and preventative programs are available, they focus on an age group where little can be done to prevent osteoporosis and have limited ability to reach the high-risk minorities.

The results of this study can be utilized in educating specific racial/ethnic groups regarding the importance of adequate dietary calcium and vitamin D, the critical role of physical activity in increasing muscle mass, strength and BMD, and the detrimental effects of excessive fat mass on bone strength. This type of research is fundamental for creating awareness, identifying the at-risk groups, and developing diagnostic criteria based on which preventative and therapeutic strategies can be established in an ethnically appropriate manner.

Assumptions

1. Participants were honest and accurate while completing the health screening questionnaire and other questionnaires.
2. Participants did an overnight fast prior to the blood draw.
3. Participants gave maximum effort during the handgrip test, jump test, and 1RM leg press test.

Delimitations

1. This study included premenopausal (18-45 years) healthy women belonging to three different races/ethnicities- Caucasians, East-Asians, and South-Asians.
2. All the participants were from the University of Oklahoma, Norman, and surrounding areas.
3. Participants with metal implants in the hip and spine were excluded.

Limitations

1. This was a cross-sectional study and hence did not develop a causal relationship.
2. Extrinsic factors like diet and genetics were not controlled.
3. Participants in the study were volunteers, and therefore, may not be an accurate representation of the entire population.

Operational Definitions

1. Bone Mineral Content: Mass of mineral contained in an entire bone (g) or mass of mineral contained in a unit length of the bone (g/cm) (Rauch & Schoenau, 2001).
2. Areal BMD: Mineral mass of the bone in a unit area, in a given direction (g/cm²) (Rauch & Schoenau, 2001).

3. Volumetric BMD: Amount of bone mineral (mg) in a cubic cm of the bone (Stagi, Cavalli, Cavalli, De Martino, & Brandi, 2016).
4. Dual-Energy X-Ray Absorptiometry (DXA): An imaging technique used to assess BMD, BMC, and body composition in children and adults using low dose ionizing radiation. It is used for diagnosing osteoporosis, fracture risk and monitoring response to treatment (Schousboe, Shepherd, Bilezikian, & Baim, 2013).
5. Cortical bone: It is also known as the compact or dense bone and constitutes 80% of the skeletal mass and has a low porosity (5-20%). It is predominantly found in the diaphysis of long bones (Morgan et al., 2013).
6. Trabecular bone: It is also known as the cancellous or spongy bone and constitutes 20% of the skeletal mass. It is primarily found in the axial skeleton and the metaphyses and epiphyses of long bones. It is highly porous, with porosity ranging from 40% in the femur neck to over 95% in the elderly spine (Morgan et al., 2013).
7. Osteoblasts: These are pluripotent cells derived from mesenchymal stem cells that mediate bone formation (Morgan et al., 2013).
8. Osteoclasts: These are cells derived from hematopoietic stem cell lineage and are capable of bone removal (Morgan et al., 2013).
9. Osteocytes: These are mechanosensory bone cells that are derived from osteoblasts. These are the most abundant cells (approximately 95%) in the mammalian bones (Morgan et al., 2013; Robling & Turner, 2009).
10. Mechanotransduction: The process by which skeletal muscle-derived mechanical forces lead to bone adaptation via initiating catabolic or anabolic events in the bone (Turner, Forwood, & Otter, 1994).

11. Osteoporosis: A condition when BMD T-score is -2.5 or below, as measured by DXA (Burge et al., 2007).
12. Osteopenia: A precursor to osteoporosis, defined by a BMD T-score between -1 to -2.5 SD, as measured using DXA (Burge et al., 2007).
13. Sarcopenia: A progressive loss of skeletal muscle mass and strength. Defined as a reduction in appendicular skeletal mass (ASM) of 2 SD or below the expected mean for healthy young adults: $\leq 7.26 \text{ kg/m}^2$ for men, and $\leq 5.45 \text{ kg/m}^2$ for women, as measured using DXA (Tae Nyun Kim & Choi, 2013).
14. Osteosarcopenic Obesity (OSO): A syndrome defined by the simultaneous existence of increased fat mass, with low bone and muscle mass (Ilich et al., 2014).
15. Premenopausal women: Women who have no symptoms of menopause and experience regular menstrual cycle most months in a year.
16. Peripheral Quantitative Computed Tomography (pQCT): An imaging technique for measuring volumetric BMD, bone strength, and geometry (Cointry et al., 2014).
17. Jump Power: An assessment of neuromuscular performance based on the product of the average force (weight of the participant multiplied by acceleration from gravity) and average velocity of weight lifted vertically (Singh et al., 2014).
18. Handgrip test: An upper limb muscle strength assessment test. Low handgrip strength is associated with a low BMD and is a clinical biomarker of muscle weakness (McGrath, Kraemer, Vincent, Hall, & Peterson, 2017).
19. 1-Repetition-Maximum (RM) test: Maximum load for which one full sequence of movement can be performed ending back in starting position (Verdijk, Van Loon, Meijer, & Savelberg, 2009).

20. Vitamin D: A potent steroid hormone, which can be synthesized by UVB rays in the skin and plays a major role in bone metabolism by controlling calcium and phosphate homeostasis (van Driel & van Leeuwen, 2017).
21. Bone Strength Indices (BSI): An estimate of bone structural stiffness in anteroposterior bending or torsion (Cointry et al., 2014).
22. Stress-Strain Index (SSI): A cortical density-weighted measure of bone structural stiffness representing both geometrical and material strength of the bone in anteroposterior bending or torsion (Cointry et al., 2014).
23. Polar Moment of Inertia (iPOLAR): Sum of the products of the area of each pixel on the cortical bone and square of its distance from the neutral axis. It represents the architectural stiffness of the bone to torsional forces (Cointry et al., 2014).
24. Cortical Area: Area of the pixels identified as cortical by the pQCT software (Cointry et al., 2014).
25. Endosteal Circumference: The circumference of the inner layer that surrounds the medullary cavity (Swinford & Warden, 2010).
26. Periosteal Circumference: The circumference of the outer layer of tissue that surrounds the bone (Swinford & Warden, 2010).
27. Muscle Cross-Sectional Area: the total area of the muscle as assessed using pQCT (Stagi et al., 2016).

CHAPTER II

REVIEW OF LITERATURE

The purpose of this review was to present the results of published literature on racial/ethnic differences in bone mineral density, bone free lean mass, fat mass and muscle strength in Caucasian, East-Asian and South-Asian women. This review has been divided into two major sections:

1. The first section highlights the mechanical relationships between the bone-muscle-fat unit, by keeping bone in the forefront. This section also elaborates on the role of vitamin D in bone metabolism.
2. The second section systematically reviews the literature on racial/ethnic differences in bone, muscle, and fat between Caucasian, East-Asian and South-Asian women, and draws attention to the limited literature available on Asian sub-groups, particularly South-Asians.

Section 1

BONE

Bone Metabolism

Bone formation begins at developmentally determined sites by replacement of the cartilage template with mineral deposits via the process of bone ‘modeling’. Bone ‘remodeling’ occurs in the adult skeleton and maintains mass and metabolic activities of the bone throughout life (Raisz, 1999). Bone remodeling is triggered via various mechanical and chemical stimuli, to which the bone responds by controlling the balance between new bone formation and local resorption of the older bone (Morgan et al., 2013).

The coupling process of bone formation and resorption is tightly controlled by the activities of three types of cells, osteoblasts, osteocytes, and osteoclasts. Osteoblasts are pluripotent cells belonging to the mesenchymal stem cell lineage which mediate bone formation, whereas, osteoclasts are derived from hematopoietic cell lineage and are capable of bone removal (Morgan et al., 2013). Following the initial activation of the bone, mononuclear osteoclast precursors differentiate into mature multinuclear osteoclasts. These cells then initiate bone resorption by secreting hydrogen ions, lysosomal enzymes, and cathepsin K, leading to degradation of the bone matrix. This is followed by bone formation, which is initiated by the proliferated osteoblasts by synthesizing an osteoid matrix over the resorption cavities. New bone formation continues until the osteoblasts gradually stop, and form quiescent surface-lining cells, which connect to the osteocytes in the bone matrix (Kini & Nandeesh, 2012).

Osteocytes are a matured osteoblast, derived from mesenchymal stem cells. Their interconnections to the surface lining cells and neighboring osteocytes permits the transmission of chemical and mechanical signals across the bone network. This interconnected signaling allows the bones to adapt to external mechanical stimuli, like those produced via muscle contraction, making osteocytes vital for mechanosensation (Morgan et al., 2013; Robling & Turner, 2009).

Osteoporosis

Any imbalance between bone formation and resorption causes biological changes in the structural and material properties of the bone, markedly declining whole bone strength and the ability of the bone to resist fractures (Morgan et al., 2013). Thus, '*Osteoporosis*' is characterized by a low bone mass and microarchitectural damage of the bone tissue, with a simultaneous increase in bone fragility and vulnerability to fractures (Kanis, 2002).

As per the World Health Organization, diagnosis of osteoporosis is based on areal bone density measurements at the lumbar spine, femoral neck, total femur and one-third radius by DXA. For postmenopausal women, a T-score of -2.5 SD or below the young reference population indicates osteoporosis and between -1 to -2.5 SD represents osteopenia, a forerunner of osteoporosis. For women prior to their menopause, Z-scores are preferred instead of T-scores, with a Z-score of -2.0 or lower being defined as “*below the expected range for age*”, and above -2.0 as “*within the expected range for age*” (Schousboe et al., 2013).

Epidemiology and Cost of Osteoporosis

As per the *Bone Health and Osteoporosis: A Report of the Surgeon General*, “*fractures due to bone diseases are common, costly, and often become a chronic burden on individuals and society*” (Wilkin, Jackson, Sims, & Haddock, 2010).

It is predicted that the U.S. population will increase from 323 million currently, to 347 million in 2025, with the majority of this increase accounted for by the rapidly increasing elderly population. This indicates an increased incidence of chronic diseases and age-related disorders in the near future. It is documented that approximately 1.5 million people suffer from osteoporosis related fractures annually, with the risk being higher in women in comparison to men. It is expected that this number will increase to more than 3 million by 2025, incurring a financial burden of 25.3 billion dollars (Burge et al., 2007).

The loss of bone mass resulting in osteoporosis may be attributable to an impairment in bone formation during remodeling, an inability to gain optimal peak bone mass during young adulthood, or increased bone resorption following attainment of peak bone mass (Raisz, 1999).

Peak Bone Mass

Peak bone mass is an important predictor of BMD and is defined as the amount of bone tissue present at the end of skeletal maturation. Although the age at which peak bone density is reached is inconclusive, most authors believe that 90% of the peak bone mass establishes within a few years following menarche and the remaining 10% is achieved by the end of the third decade of life (Lu, Shin, Yen, & Sun, 2016). Although 60-80% of the variation in BMD is controlled by genetic factors, inadequate non-genetic modifiable factors, such as physical activity, and calcium and vitamin D intake can limit the achievement of an optimal peak bone mass and increase the risk of fractures later in life (Heaney et al., 2000). In a longitudinal study by Lu et al, peak BMD values achieved by individuals were highly correlated to the predicted BMD values of the same individuals as adults ($r=0.96$; $p<0.0001$). Hence, this study supports the results of prior literature in which a high degree of tracking in BMD has been reported from childhood into early adulthood. This indicates the importance of achieving optimal BMD during young adulthood to reduce the risk of osteopenia/osteoporosis in later life (Lu et al., 2016). Contrary to this, prior research has mostly emphasized the understanding of bone health and its determinants in postmenopausal women, when the clinical changes cannot be reversed.

BONE-MUSCLE INTERACTION

Mechanotransduction

A critical factor in the development and maintenance of bone mass is skeletal muscle-derived mechanical loading. The tight coupling between the musculoskeletal system is recognizable right from embryonic development, through growth and aging (Bonewald, 2019). Muscle contractions contribute to the development of the skeleton during intra-uterine life and muscle and bone growth postnatally (Brotto & Bonewald, 2015).

The mechanism for adaptation of the bone to skeletal muscle induced mechanical loading postnatally was introduced in the '*mechanostat theory*' by Harold Frost (Frost, 2000). Frost proposed that addition or removal of the bone from the skeleton was governed by certain mechanical thresholds. Thus, mechanical loading of the bone above a typical threshold, *modeling threshold*, will increase bone strength, *formation modeling*, whereas strains below a certain threshold, *remodeling threshold*, will result in 'disuse mode', eventually leading to permanent removal of the bone, *resorption remodeling*. However, loading above the remodeling threshold will stop or reduce these losses due to 'conservation mode' remodeling, thus preventing or slowing the progression of osteoporosis. Bone remodeling involves the coupled action of osteoblasts and osteoclasts, the *effector cells* of the mechanostat, where osteoclasts resorb a small patch of the bone, and osteoblasts subsequently form a nearly equal amount of bone in that area (Frost, 2000; Hughes & Petit, 2010).

Although the mechanostats' effector cells have been well characterized, the sensory cells- osteocytes, have received considerable attention lately. Osteocytes have a widespread network spanning across the cortical and trabecular compartments of the bone. Moreover, they have long cellular processes to connect with neighboring osteocytes and the surface-lining cells, thus facilitating cell to cell communication, making them perfect as a sensory type cell (Hemmatian, Bakker, Klein-Nulend, & van Lenthe, 2017). Moreover, deformation of the bone due to mechanical strains creates fluid pressure gradients in the interstitial fluid surrounding the osteocytes. This causes movement of the interstitial fluid from areas of compression to areas of tension and initiates a cascade of biochemical events that result in the gain of bone mass if the magnitude of the mechanical stimulus is above the threshold for the bone (Hughes & Petit, 2010).

Along with an increase in bone mass, increased mechanical loading by virtue of weight-bearing exercise also improves bone structure during growth leading to greater periosteal apposition. As bone loss during menopause and aging mostly occur on the endosteal surface, greater periosteal bone deposition during growth persists throughout life and increases the resistance of the bone to external loads (Warden & Fuchs, 2009). Thus, weight-bearing exercise increases bone mass as well as optimizes bone structure before skeletal maturity is reached, following which it helps to maintain bone mass during adulthood, and helps to protect the skeleton from external loads and falls later in life (Figure 3).

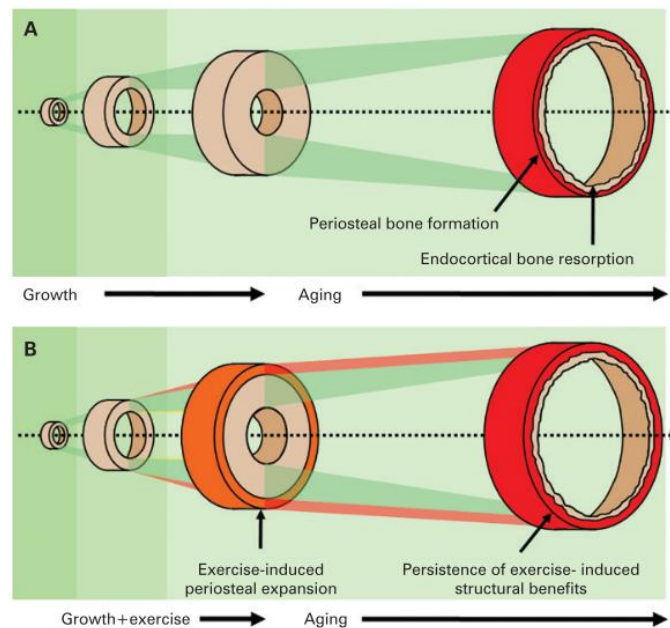


Figure 3. Structural changes associated with aging, with (A) and without (B) exercise. Periosteal apposition is preserved even after aging-induced bone loss (Adapted from Warden et al., 2009).

Sarcopenia

Both muscle and bone mass, along with their strength and quality, begin decreasing towards the end of the third decade of life (Kim et al., 2009). There are several working definitions of sarcopenia, due to which the prevalence of sarcopenia varies substantially. Baumgartner et al, first described sarcopenia as a reduction in appendicular skeletal mass (ASM) divided by height squared ($ASM/height^2$) of 2 SD or below the expected mean for healthy young adults, $\leq 7.26\text{kg}/\text{m}^2$ for men, and $\leq 5.45\text{kg}/\text{m}^2$ for women, as measured using DXA. However, this index was highly related to body mass index and thus underestimated the prevalence of sarcopenia in obese individuals. This limitation was overcome by Newman et al, and Delmonico et al, who defined sarcopenia based on the amount of lean mass being lower than that expected for a given amount of fat mass, using residuals from linear regression models. Additionally, Janssen et al, proposed sarcopenia as a Skeletal Muscle Mass Index (%) [skeletal muscle mass (kg)/weight(kg) X 100], of 1 or 2 SD below the mean of younger reference group, using bio-electrical impedance analysis (Kim et al., 2011; Kim et al., 2009).

Muscle mass does not entirely account for muscle strength, and so the more recent definition of sarcopenia incorporates both quantitative (muscle mass) and qualitative (muscle strength) declines. The AWGS (Asian Working Group on Sarcopenia) and the EWGSOP (European Working Group on Sarcopenia in Older People) assessed strength measures for defining sarcopenia in addition to muscle mass. A gait speed equal to or lower than 0.8m/s, and a handgrip strength lower than 26kg in men and 18kg in women (AWGS) were used for diagnosing sarcopenia, in addition to muscle mass (Growing research on sarcopenia in Asia, 2014).

BONE-FAT INTERACTION

Research advances have led to the understanding that apart from muscle mass and strength, bone metabolism is also integrated with adipose tissue (Kawai, Paula, & Rosen, 2012). Conventionally, excessive fat mass is linked to higher bone strength and lower fracture risk. This is attributable to the increased mechanical loading of the skeleton as a consequence of increased body weight. These increased mechanical strains due to a higher body weight associated with increased fat mass, are considered responsible for increasing bone density, particularly cortical. Additionally, fat cells express cytochrome P450 enzyme, aromatase, which generates estradiol from testosterone that helps to maintain bone mass (Rosen & Bouxsein, 2006). However, it has been reported that positive correlations between bone and fat mass disappear in Chinese and Caucasian subjects after adjusting for the mechanical loading effects of body weight. Thus, following adjustment for mechanical loading, the positive relationship between bone and fat can diminish or become negative (Yoo et al., 2012).

Initially assumed as being different, osteoporosis and obesity are now thought of being similar as they both are influenced by genetic and environmental factors, are considered pediatric conditions which manifest later in life, and are related with significant morbidity and mortality (Rosen & Bouxsein, 2006; Wilkin et al., 2010). Obesity-related risk factors and diseases that were earlier prevalent only in adults are now recognized in the younger population (Caprio et al., 2008). Excessive body weight or obesity, although a multifactorial condition, is most commonly related to excessive energy consumption and low levels of physical activity. Obesity leads to a state of low-grade chronic inflammation causing increased release of pro-inflammatory cytokines such as tumor necrosis factor- α , interleukin-6, and imbalances in the hormonal milieu, leading to loss of both muscle and bone tissues (Ilich et al., 2014; Ormsbee et al., 2014).

Bone, muscle, and fat also interact at the cellular levels. Human mesenchymal stem cells (MSC) within the bone marrow can differentiate into chondrocytes, myocytes, adipocytes, and osteoblasts (Bermeo, Gunaratnam, & Duque, 2014). Under normal conditions, MSCs slightly favor adipogenesis over osteogenesis. Various local, systemic and environmental factors, like physical inactivity or immobilization, metabolic disease, estrogen withdrawal, and glucocorticoid treatment, promote adipogenesis and prevent osteoblastogenesis. This may eventually result in a commitment dysfunction in the MSC lineage favoring adipogenesis instead of osteogenesis, thus leading to a lack of bone formation by decreasing the availability of mature osteoblasts (Ilich et al., 2014). Thus, excessive fat mass enhances these adipogenic signals and leads to loss of both bone and muscle mass.

BONE-MUSCLE-FAT INTERACTION

Osteo-Sarcopenic obesity

Bone mineral density is known to increase linearly with an increase in muscle mass and is reported to be greater and similar in high muscle/low fat and high muscle/high fat body types. However, an increase in fat mass without a concurrent increase in muscle mass results in decreased BMD. This triad of low bone mass and muscle mass, accompanied by or in the presence of high fat mass is termed as '*Osteo-Sarcopenic Obesity*' (Ilich et al., 2014; Ormsbee et al., 2014). To our knowledge, there are a handful of studies determining osteosarcopenic obesity (OSO) in elderly women, and only one study, by Stefanaki et al. (2016), reporting the prevalence of OSO in young individuals, 18-21 years old. They observed a pattern similar to that seen in OSO in middle-aged and elderly, a decreased fat-free mass in healthy overweight/obese subjects in comparison to healthy lean subjects ($p < 0.001$).

The phenomenon of OSO indicates that a derangement between muscle and fat tissues within the bone-muscle-fat unit can lead to early or rapid bone loss, resulting in earlier diagnosis of osteoporosis. As discussed in section 2 of this review, some racial/ethnic groups may have greater propensity towards a higher fat mass or lower lean mass or both, by virtue of their lifestyle and genetic makeup. This may eventually result in early or rapid loss of bone mass and in part help to explain the increased susceptibility of some ethnic groups to osteoporotic fractures in comparison to others.

BIOCHEMICAL MARKERS

While measurements based on DXA and pQCT provide an overview of skeletal status (aBMD, vBMD) and body type (fat mass, BFLBM, BMC), measuring the serum concentrations of the biochemical markers is essential further understand the relationships among bone, muscle, and fat.

Vitamin D

Research on vitamin D has been done through decades, since its discovery as an antirachitic factor, then a vitamin, and finally a potent hormone (Feldman et al., 2013). McCollum demonstrated the presence of vitamin D in cod liver oil and deemed it responsible for healing rickets and mineralization of the skeleton. Contrastingly in 1919, Huldshinsky discovered that rickets could be cured by ultraviolet light in children. Eventually, Steenbock and Black, and Hess and Weinstock, independently discovered the formation of vitamin D from ultraviolet B (UVB) radiation (DeLuca, 2008). Although natural food sources of vitamin D are limited, it can be synthesized endogenously in the exposed skin by using the energy of sunlight, distinguishing vitamin D from actual vitamins (Feldman et al., 2013).

UVB rays (290-315nm) cleave the carbon ring of provitamin molecules, 7-dehydrocholesterol, and ergosterol, at carbon- 9 and 10, to open the ring forming a ‘split’ or secosteroid structure, vitamin D₃ (cholecalciferol- animal form) and vitamin D₂ (ergocalciferol- plant form) respectively. Vitamin D₃ is biologically more potent than vitamin D₂. Both vitamin D₂ and D₃ are then hydroxylated in the liver to form the circulating metabolite, 25-hydroxy vitamin D (25OHD), followed by hydroxylation in the kidney to form the active hormone, 1,25(OH)₂D. 25OHD is commonly assayed to assess vitamin D levels in humans. Both the circulating as well as the active form of vitamin D travel in the blood bound to vitamin D binding protein (DBP). 1,25(OH)₂D then binds to its nuclear receptors, vitamin D receptor (VDR), on multiple target tissues to regulate gene expression (Ceglia & Harris, 2013; Feldman et al., 2013).

Interference with the above-mentioned processes or disruption of the small bowel mucosa can lead to malabsorption of vitamin D, resulting in vitamin D insufficiency/deficiency (Ceglia & Harris, 2013; Feldman et al., 2013). Apart from this, the sequestration of lipophilic vitamin D metabolites in the adipose tissue can also result in vitamin D insufficiency/deficiency in obese individuals (Feldman et al., 2013). Vitamin D deficiency is defined as serum 25OHD level below 20ng/ml, and vitamin D insufficiency as less than 30ng/ml (75nmol/liter) (Gallagher & Sai, 2010). Considering the enormous capacity of the skin to produce vitamin D, factors altering exposure to sunlight can dramatically affect the synthesis of vitamin D. The amount of solar radiation can be limited by changes in the zenith angle, decreases with increasing global latitude- especially in countries above 40 degrees latitude north and south of equator, and during fall and winter months, when the sun is lower in the sky. Similarly, a darker skin color i.e. increased melanin content, protects the body from excessive sunlight by acting as a competitor

of 7-dehydrocholesterol for UVB rays, thus decreasing the amount of UVB available for the synthesis of vitamin D. This reduces the efficiency of formation of cholecalciferol. Sunscreens also work just like melanin, however, their effectiveness varies on the frequency of application and their strength. Additionally, non-modifiable factors like advancing age decrease the amount of 7-dehydrocholesterol available in the skin and its efficiency to form cholecalciferol (Feldman et al., 2013; Pfeifer, 2002).

In the human body, the most classic role of vitamin D is to regulate bone metabolism through homeostatic control of calcium and phosphate. In response to slight hypocalcemia, parathyroid hormone (PTH) secretion is stimulated. PTH binds to the epithelial cells of the renal tubules stimulating the CYP27B1 gene, producing the 1α -hydroxylase enzyme that synthesizes $1,25(\text{OH})_2\text{D}_3$, which then activates the three sites required for calcium mobilization. It enhances calcium and phosphate absorption in the intestine. When dietary calcium levels are low, calcium retention is increased from the distal tubules of the kidneys, and via osteoclast-mediated bone resorption from the skeleton (van Driel & van Leeuwen, 2017).

The direct effects of vitamin D on the osteoblasts are observed by the binding of vitamin D with the VDRs located on the osteoblasts. Upon binding, the VDR heterodimerizes with Retinoid X Receptor (RXR), which ultimately binds to the DNA to regulate gene expression. This vitamin D activated VDR is also known to increase expression of Wnt coreceptor, LRP5, thus increasing anabolic effects on bone (Feldman et al., 2013; van Driel & van Leeuwen, 2017).

Vitamin D deficiency has also been associated with reversible myopathies in clinical settings, along with reduced muscle mass, performance, strength and increased risk of falls. VDRs have been identified in muscle tissue, and they appear to decline with age (Dawson-

hughes, 2019; Pojednic & Ceglia, 2014). Large interfibrillar spaces and fat infiltration is also reported in muscle cross-sections of individuals with vitamin D deficiency. Moreover, profound vitamin D deficiency is also associated with atrophy of type II muscle fibers, a pattern similar to that seen in aging. The NHANES study reported an inverse association between serum 25OHD levels and timed-walk and sit-to-stand tests, with a steep decline at low and low-normal 25OHD levels (Dawson-hughes, 2019). Stockton, Mengersen, Paratz, Kandiah, & Bennell, 2011, analyzed 17 randomized controlled trials evaluating the influence of vitamin D on muscle performance and concluded that vitamin D supplementation did not significantly affect muscle strength in adults. However, a meta-analysis by Beudart et al. (2014), observed a slightly significant improvement in muscle strength with vitamin D supplementation, with no apparent effect on muscle mass.

With multiple target sites and risk factors, including skin pigmentation, age, latitude, physical activity, diet, sun exposure, season, the assumption that serum 25OHD levels will vary as per gender and race/ethnicity seems to be reasonable. In places with ample sunlight (UVB) reaching the earth's surface, like the Indian sub-continent, located between 8.4 degrees north and 37.6 degree north, Indians can be assumed as having adequate vitamin D levels due to sufficient sunlight available for cutaneous vitamin D synthesis. However, a study conducted on young hospital staff in an urban city of India reported decreased 25OHD concentration in 66.3% of the volunteers. This may be primarily attributable to lack of vitamin D rich diet, absence of vitamin D fortified foods, lack of sun exposure due to indoor confinement, darker skin color, clothing covering maximum part of the body except for hands and face (Arya, Bhambri, Godbole, & Mithal, 2004). Not only adults, but deficiency of vitamin D was also reported in apparently healthy school children from northern India, who spent approximately 30 minutes in

the sun daily. From the 760 school children, 35.7% had 25OHD levels <9ng/ml (Marwaha et al., 2005).

Alekel et al. (1999), conducted a study on Indian/Pakistani women residing in the U.S. It was concluded that premenopausal Indian/Pakistani women had lower vitamin D concentration, and a lower proximal femur BMD as compared to Caucasian women. Similarly, in comparison to Chinese (52.3%), Asian-Indians (84.3%) had a higher prevalence of sub-optimal 25OHD levels ($\leq 29\mu\text{g/L}$) ($p < 0.001$). While numerous studies have assessed vitamin D levels separately in Asian sub-groups, comparison of these studies to assess prevalence and risk factors of vitamin D in different ethnicities becomes difficult, as different studies have different cut-off values for vitamin D deficiency/insufficiency. A cut-off value of 20ng/ml was used for defining vitamin D deficiency by Babu & Calvo, 2010, whereas Lu et al. (2018), used a cut-off value of approximately 30ng/ml.

Section 2

RACIAL/ETHNIC DIFFERENCES

This section will systematically review the results of published literature regarding racial/ethnic differences in bone mineral density, lean mass, fat mass and muscle strength between Caucasian, East-Asian and South-Asian women. The review was summarized qualitatively, and meta-analysis was not performed due to variations between the methodologies used in different studies.

A literature search was conducted using PubMed for all articles in English language published till May 17, 2019, using keywords, *race AND bone mineral density; ethnicity AND bone mineral density; Asian AND bone mineral density; race AND lean mass; ethnicity AND lean*

mass; Asian AND lean mass; race AND fat mass; ethnicity AND fat mass; Asian AND fat mass; race AND muscle strength; ethnicity AND muscle strength; Asian AND muscle strength.

Articles were included if they examined areal BMD, lean mass and fat mass using DXA, and muscle strength in Asian and Caucasian adult women (≥ 18 years) residing in the United States. Apart from Asians and Caucasians, studies could include other ethnicities or males, however, only the data from Asian and Caucasian females will be reported in this review.

Review articles and studies testing the efficacy of dietary, exercise or medical interventions were not included in the review. Additionally, studies including participants taking medications that could affect bone and muscle metabolism or those testing clinical populations such as participants with diabetes mellitus, renal disorders, cardiovascular disorders or any other physical disabilities were excluded from the review. Table 1 outlines the inclusion and exclusion criteria for the selected articles and Figure 4 depicts the number of retrieved articles and the selection strategy.

Table 1: Criteria for inclusion/exclusion of studies.

	Inclusion	Exclusion
Population	Asian and Caucasian adult women (≥ 18 years) residing in United States.	Women taking medications affecting bone and muscle metabolism or clinical populations such as participants with diabetes mellitus, renal disorders, cardiovascular disorders or any other physical disabilities.
Intervention	Studies evaluating areal BMD at the lumbar spine, or femoral neck or both using DXA; evaluating body composition using DXA; testing muscle strength using muscle strength tests.	Studies evaluating the effects of medications on bone and muscle, or those including structured exercise programs targeted to increase bone and muscle health.
Comparison of interest	Comparing Caucasians with Asians.	N/A
Outcomes	Areal BMD, body fat percentage, fat mass, lean body mass, muscle strength.	N/A
Study design	Cross-sectional studies comparing Caucasians and Asians. Studies can include other racial/ethnic groups; however, this review will only focus on comparison between Caucasians and Asians.	Longitudinal studies, studies using data from another study or manufacturer provided values as reference database, editorials, reviews, comments, and studies published only as abstracts, posters, and dissertations.

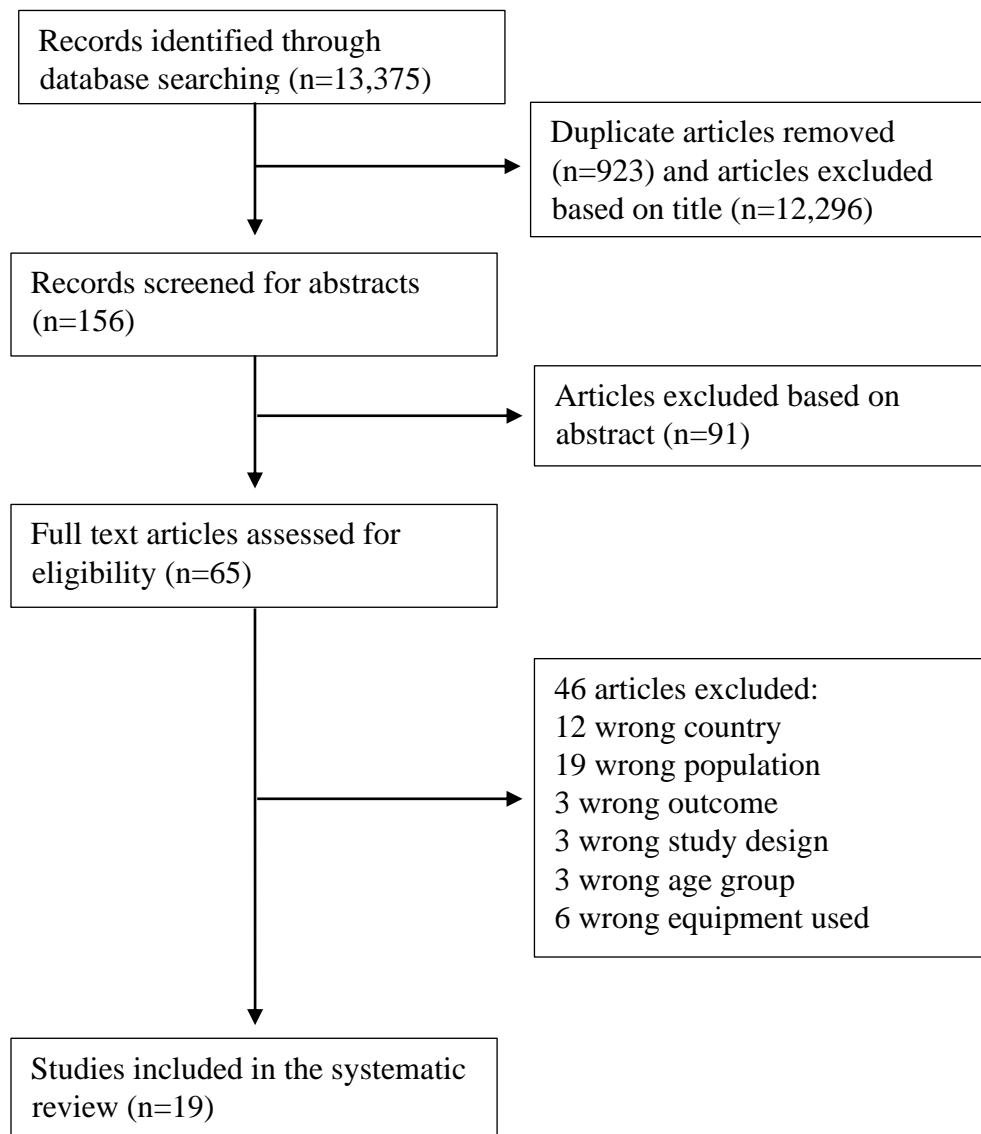


Figure 4. Flow diagram for literature search and selection strategy.

Racial/Ethnic Differences in Bone Mineral Density

Although the relative contributions of genetic and environmental components to bone density remain uncertain, the twin and family studies have shown high heritability of BMD and fracture risk. At least 15 genes have been designated as osteoporosis susceptibility genes, with over 30 more genes assigned as promising candidate genes (Duncan & Brown, 2008).

DNA genotyping of postmenopausal women, who self-reported as being either African-American or non-Hispanic White, revealed that hip geometry and BMD at the hip and femoral neck were significantly ($p < 0.05$) higher in women with higher percentage of African admixture ($> 50\%$). The authors concluded that higher African admixture in African-American women relative to Europeans resulted in higher bone strength, despite a greater rate of bone loss following menopause. These strength advantages were linked to a significantly higher peak bone mass in African women (Chen et al., 2011). This study signifies that self-reported racial/ethnic categorization used in most bone health studies is well suited to assess BMD, peak bone mass, fracture risk and their interaction with environmental risk factors such as nutrition and physical activity.

Tables 2 and 3 outline the variables measured and the outcomes for studies assessing BMD in Caucasians and Asians. Bone mineral density at the lumbar spine is reported to be higher in Caucasians (0.939 (0.921 – 0.957) g/cm^2) than Filipinas (0.896 (0.876 – 0.916) g/cm^2 ; $p < 0.001$) after adjustment for covariates (Morton, Barrett-Connor, Kritiz-Silverstein, Wingard, & Schneider, 2003). However, Davis et al. (1994), compared bone mass among Hawaiian, Filipino, Japanese and White women and concluded that lumbar spine BMD was higher in White (1.04 ± 0.12 g/cm^2) than in Filipino women (0.99 ± 0.10 g/cm^2) only before controlling for covariates. After adjusting for height and weight, most of these differences were eliminated

and BMD remained significantly lower only at the distal radius in White women, and this was attributable to their narrower bone width in comparison to Filipino and Japanese women, and lower bone mineral content compared to Hawaiian women. Unlike at the distal radius, proximal femur and femoral neck BMD is reported to be lower in both Chinese and Japanese women in comparison to White women (Ishii et al., 2012; Nakamura et al., 1994). Consistent with these findings, other studies have reported similar or lower BMD at the lumbar spine, forearm and femoral neck in Asians, particularly Chinese, compared to Caucasian women (Boutroy et al., 2014; Fielding et al., 2002; Liang et al., 2007; Walker et al., 2011; Marquez et al., 2001; Silva et al., 2013).

Contrary to this, a large cross-sectional study assessing racial/ethnic differences in BMD, the Study of Women's Health Across the Nation (SWAN), reported that lumbar spine and femoral neck BMD was higher in African-Americans in comparison to Caucasian, Chinese and Japanese women ($p < 0.001$ for comparison of African-Americans with all other groups). When a subset of women weighing less than 70kg was analyzed separately, FN BMD remained higher in African-American women compared to the other groups ($p \leq 0.001$ vs. Caucasians and Japanese; $p = 0.071$ vs. Chinese), while LS BMD was higher in African-American and Chinese than in Caucasian women ($p = 0.003$, $p = 0.008$, and $p = 0.072$ for comparison of Caucasians with African-American, Chinese and Japanese women respectively). This study concluded that differences in BMD after adjustment of covariates can be linked to ethnic variation in genes associated with BMD (Finkelstein et al., 2002). Similarly, studies by Walker et al. (2009), and Liu et al. (2011) reported a higher BMD at the lumbar spine, femoral neck and total hip in Chinese-American than in Caucasian women ($p < 0.01$).

Regardless of the BMD values, the resistance offered by the femoral neck to mathematically modeled forces mimicking a hypothetical fall was 40% greater in Japanese than in White women (Nakamura et al., 1994). Additionally, both Chinese and Japanese women also had greater values for composite indices of femoral neck strength (compression strength index; bending strength index; impact strength index) than Caucasians ($p < 0.01$). Within Japanese, foreign-born Japanese had higher values for composite strength indices than US-born Japanese ($p < 0.01$). Considering that both U.S. born and foreign-born Japanese have similar genetic make-up, these differences were primarily believed to be related to differences in lifestyle and health behaviors between the U.S. and foreign-born Japanese (Ishii et al., 2012).

In comparison to East-Asian women such as Chinese and Japanese, studies investigating BMD and its predictors in South-Asian are limited and not well defined. Studies done in South-Asian countries, like India, report that premenopausal Indian females (20-30 years) have a significantly lower BMD (g/cm^2) at the femur neck (0.871 ± 0.091 vs. 0.956 ± 0.120 ; $p = 0.0001$) and lateral spine (0.654 ± 0.108 vs. 0.768 ± 0.120 ; $p = 0.0001$) in comparison to the manufacturer provided values for Caucasians. Although non-significant, postmenopausal Indian women (61-70 years) had lower femur neck BMD (0.750 ± 0.115 vs. 0.801 ± 0.120) and higher lateral spine BMD than the manufacturer provided values for Caucasians (0.508 ± 0.152 vs. 0.443 ± 0.120 ; $p = 0.0001$). The authors postulated the measurement of lateral spine BMD, instead of the anteroposterior spine, maybe a potential factor for incongruence of these values (Makker et al., 2008). Moreover, Alekel et al. (1999), reported that proximal femur (0.875 ± 0.096 vs. 0.937 ± 0.088 ; $p = 0.0014$) and trochanter (0.652 ± 0.082 vs. 0.705 ± 0.073 ; $p = 0.0013$) BMD (g/cm^2) was lower in Indian/Pakistani women than in Caucasian women residing in United States.

Table 2. Description of the variables measured

Author and year	Sample size	Age	Variables measured
(Davis, Novotny, Ross, & Wasnich, 1994)	Hawaiian=66 White=137 Japanese=144 Filipino=74	25-34 years	Assessed LS BMD.
(Nakamura et al., 1994)	Japanese=57 White=119	50-79 years	Assessed proximal femur BMD
(Alekel et al., 1999)	Caucasian=47 Indian/Pakistani=47	20-40 years	Assessed BMD at the proximal femur. Body composition was measured using DXA.
(Davis, Novotny, Wasnich, & Ross, 1999)	White=9689 Japanese=690	≥ 65 years	Assessed maximum grip strength in the dominant hand, isometric strength for quadriceps and triceps strength.
(Marquez et al., 2001)	White= 349 Southeast Asian=240	≥ 20 years	Assessed BMD at the lumbar spine (L1-4) and proximal femur.
(Fielding et al., 2002)	Asian=144 African-American=323 Latina=129 Caucasian=308	19-26 years	Assessed BMD at the lumbar spine (L1-4), left proximal femur, and total body.
(Finkelstein et al., 2002)	Caucasians=1051 Chinese=232 Japanese=257 African-American=591	42-52 years	Assessed BMD at the lumbar spine (L1-4) and femoral neck.

Table 2. Description of the variables measured (continued)

Author and year	Sample size	Age	Variables measured
(Morton et al., 2003)	Caucasians=354 Filipinas=285 Hispanics=168	50-69 years	Assessed BMD at the lumbar spine (L1-4), total hip, femoral neck, and total body. Body composition was measured using DXA.
(Sun et al., 2003)	European-American (non-Hispanic Caucasian)= 227 African-American=128 Asian-American=89	20-94 years	Body composition was measured using DXA.
(Liang et al., 2007)	Asian=40, Caucasian=36 Hispanic=39	20-35 years	Assessed BMD at the forearm (radius and ulna), lumbar vertebrae 1-4, femoral neck, greater trochanter, lower extremity (femoral neck to foot) and whole body. Body composition was measured using DXA and bilateral leg muscle strength was assessed using supine leg press machine.
(Walker, McMahon, Udesky, Liu, & Bilezikian, 2009)	Chinese American=31 White=32	29-40 years	Assessed BMD at the lumbar spine (L1-4), total hip, femoral neck, and 1/3 radius. Volumetric BMD and microarchitecture were assessed at the non-dominant distal radius and tibia.

Table 2. Description of the variables measured (continued)

Author and year	Sample size	Age	Variables measured
(Liu et al., 2011)	White=46 Chinese American=49	29-40 years	Assessed BMD at the lumbar spine (L1-4), total hip, femoral neck, and 1/3 radius. Bone microarchitecture, Individual trabecula segmentation (ITS), and micro finite element analysis were assessed at the non-dominant distal radius and tibia.
(Walker et al., 2011)	White=68 Chinese American=29	58-69 years	Assessed BMD at the lumbar spine (L1-4), total hip, femoral neck, and 1/3 radius. Volumetric BMD, bone microarchitecture, and finite-element analysis were assessed at the non-dominant distal radius and tibia.
(Ishii et al., 2012)	Caucasian= 968 African- American=512 Japanese= 239 Chinese= 221	42-53 years	Assessed FN BMD and composite indices of femoral neck strength.
(Khandewal, Chandra, & Lo, 2012)	South-Asian= 449 Chinese=2245 Whites=4490	50-85 years	Assessed FN BMD
(Danielson et al., 2013)	Caucasian=966 African- American=517 Chinese=220 Japanese=239	42-52 years	Assessed BMD at the femoral neck and lumbar spine.

Table 2. Description of the variables measured (continued)

Author and year	Sample size	Age	Variables measured
(Silva et al., 2013)	Chinese-American=57 White=58	Premenopausal=29-40 years Postmenopausal=59-70 years	Assessed BMD at the lumbar spine (L1-4), total hip, femoral neck, and 1/3 radius. Volumetric BMD and microarchitecture were assessed at the non-dominant distal radius and tibia.
(Walker et al., 2014)	Chinese-American=70 Caucasian=76	Premenopausal=29-40 years Postmenopausal=59-70 years	Assessed BMD at the lumbar spine (L1-4), femoral neck and 1/3 radius. Bone microarchitecture and Individual trabecula segmentation (ITS) was assessed at the non-dominant distal radius and tibia.
(McGrath, Ottenbacher, Vincent, Kraemer, & Peterson, 2017)	Ethnicity (%): Non-Hispanic white=47.8 Non-Hispanic black=19.6 Hispanics=21.7 Non-Hispanic Asian=10.9	≥ 40 years	Assessed BMD at the proximal femur. Bilateral handgrip strength was tested.

Table 3. Outcome measures for bone mineral density

Author and year	Outcome for bone mineral density
(Davis et al., 1994)	LS BMD (g/cm^2) was higher in White (1.04 ± 0.12) than in Filipino (0.99 ± 0.10) women ($p < 0.05$).
(Nakamura et al., 1994)	Proximal femur BMD (g/cm^2) was lower in Japanese (0.70 ± 0.13) than in White (0.82 ± 0.11) women.
(Alekel et al., 1999)	Proximal femur (0.875 ± 0.096 vs. 0.937 ± 0.088 ; $p = 0.0014$) and trochanter (0.652 ± 0.082 vs. 0.705 ± 0.073 ; $p = 0.0013$) BMD (g/cm^2) was lower in Indian/Pakistani women than in Caucasian women.
(Marquez et al., 2001)	Southeast Asians have a lower BMD (g/cm^2) at the LS (PRE: 1.014 ± 0.1 vs. 1.096 ± 0.1 ; POST: 0.824 ± 0.1 vs. 0.948 ± 0.2) and FN (PRE: 0.787 ± 0.1 vs. 0.865 ± 0.1 ; POST: 0.639 ± 0.1 vs. 0.679 ± 0.1) than Whites ($p < 0.001$).
(Fielding et al., 2002)	No differences in LS, left proximal femur, and whole-body BMD between Asians and Caucasians.
(Finkelstein et al., 2002)	In a subset of women weighing less than 70kg, LS BMD was higher in Chinese than in Caucasian women ($p = 0.008$). Chinese had higher LS and FN BMAD than Caucasians ($p = 0.021$ and 0.015 respectively).
(Morton et al., 2003)	LS BMD (g/cm^2) was higher in Caucasians (0.939 (0.921 – 0.957)) than Filipinas (0.896 (0.876 – 0.916)); $p < 0.001$).
(Liang et al., 2007)	Asians had lower BMD (g/cm^2) at the forearm (0.673 ± 0.042 vs. 0.718 ± 0.048) and femoral neck (0.786 ± 0.120 vs. 0.870 ± 0.102) in comparison to Caucasians ($p < 0.05$), however, after adjusting for covariates the differences were not significant.
(Walker et al., 2009)	BMD (g/cm^2) at the LS (11.058 ± 0.120 vs. 0.975 ± 0.111 ; $p < 0.03$), FN (0.831 ± 0.111 vs. 0.757 ± 0.104 ; $p < 0.05$), TH (0.963 ± 0.116 vs. 0.866 ± 0.108 ; $p < 0.01$) was higher in Chinese American than in White women.

Table 3. Outcome measures for bone mineral density (continued)

Author and year	Outcome for bone mineral density
(Liu et al., 2011)	As measured by DXA, LS BMD (g/cm^2) was higher in Chinese American (1.027 ± 0.142) than in White women (1.020 ± 0.120 ; $p < 0.05$).
(Walker et al., 2011)	No significant differences in BMD as measured by DXA at the LS, TH, FN, and 1/3 of radius.
(Ishii et al., 2012)	Chinese (0.77 ± 0.10) and Japanese (0.76 ± 0.096) had lower BMD (g/cm^2) at the femoral neck than Caucasians (0.83 ± 0.12 ; $p < 0.001$).
(Khandewal et al., 2012)	For women aged 50-59 years, South-Asians (0.75 ± 0.11) and Whites (0.75 ± 0.11) had a higher FN BMD (mg/cm^2) than Chinese (0.72 ± 0.11). For 60-69-year-old women, FN BMD was higher in South-Asians (0.70 ± 0.12) and Whites (0.72 ± 0.12) than in Chinese (0.67 ± 0.11), and higher in Whites than in South-Asians. For women above the age of 70 years, FN BMD was higher in Whites (0.67 ± 0.11) than in Chinese (0.63 ± 0.11 ; $p < 0.01$).
(Danielson et al., 2013)	No significant difference in LS and FN BMD.
(Silva et al., 2013)	FN (0.77 ± 0.081 vs. 0.824 ± 0.116) and TH (0.895 ± 0.086 vs. 0.947 ± 0.129) BMD was lower in pre-menopausal Chinese-American women than Caucasian women ($p < 0.05$). There were no racial differences for BMD in post-menopausal women and trabecular bone score values for both pre- and post-menopausal women.
(Walker et al., 2014)	No differences in BMD between the two groups at the LS, FN, TH, and one-third radius.

The differences in BMD were attributable to maximum non-pregnant weight achieved during the lifetime (kg), which was a significant contributor to BMD ($p < 0.0003$) and was higher for Caucasian than Indian/Pakistani women (65.0 ± 8.2 vs. 58.7 ± 9.5 ; $p = 0.0008$). In contrast to these results, Khandewal et al. (2012) reported that for women aged 50-59 years, South-Asians (0.75 ± 0.11) and Whites (0.75 ± 0.11) had a higher FN BMD (mg/cm^2) than Chinese (0.72 ± 0.11). Similarly, for 60-69-year-old women, FN BMD was higher in South-Asians (0.70 ± 0.12) and Whites (0.72 ± 0.12) than in Chinese (0.67 ± 0.11), and higher in Whites than in South-Asians, however, for women above the age of 70 years, FN BMD was higher in Whites (0.67 ± 0.11) than in Chinese (0.63 ± 0.11 ; $p < 0.01$). However, their ability to accurately interpret the results was vastly limited by the scarce and inconclusive BMD database in South-Asians.

Racial/Ethnic Differences in Lean Mass, Fat Mass, and Muscle Strength

Considering the close relationship between muscles and bones, and the contribution of genetics (approx. 45%) to metabolic profile and fiber composition, it is reasonable to assume that muscle mass and strength, like bones, are influenced by age as well as racial/ethnic background (Suminski, Mattern, & Devor, 2002). Liang et al. (2007), compared lean mass, fat mass, and muscle strength in young women (20-35 years) belonging to Caucasian, Asian and Hispanic backgrounds. This study reported that (Table 4) total body lean mass and fat mass (kg) were lower in Asians (lean mass: 36.3 ± 3.94 , fat mass: 15.2 ± 20.8) than in Caucasian women (lean mass: 43.4 ± 6.67 , fat mass: 20.8 ± 7.97 ; $p = 0.01$). Leg muscle strength measured using the 1RM leg press test was a strong predictor of lower extremity BMD in Caucasian, Hispanic and Asian women (multiple $R = 0.401$ to 0.647). Further, leg strength (lb) was highest

in Caucasians, followed by Hispanics and least in Asians ($214 \pm 46.4 > 191 \pm 44.2 > 161 \pm 39.3$; $p=0.01$) (Liang et al., 2007).

Consistent with these findings, triceps (10.5 ± 2.7 vs. 9.38 ± 2.12) and quadriceps muscle (67.8 ± 27.6 vs. 35.7 ± 17.2) strength (kg) has been reported to be higher in White than in Japanese women ($p < 0.05$) (Davis et al., 1999). In another study comparing muscle strength across different ethnicities- non-Hispanic blacks, non-Hispanic whites, non-Hispanic Asians and Hispanics, an increase in handgrip strength was associated with reduced odds of osteoporosis in both men and women. The odds of osteoporosis were lowest for non-Hispanic blacks and highest for non-Hispanic Asians ($p < 0.0001$) (McGrath, Kraemer, et al., 2017). Although the relationship between muscle strength and bone mass is well established, it remains relatively unclear how this relationship will vary as per race/ethnicity. Quantification of muscle strength using grip force, jump test, dynamometry, electromyography, will provide more accurate estimates than using muscle mass alone. Differences in muscle force and power, and their relationship to BMD, may partially help to explain the ethnic differences in fracture rates (Zengin, Prentice, & Ward, 2015).

Similar to Liang et al. (2007), it has been previously reported that total fat mass (23.1 (22.3 – 24.0) vs. 19.8 (18.9 – 20.7) kg) and total lean mass (40.4 (39.9 – 40.9) vs. 36.7 (36.3 – 37.5) kg) was higher in Caucasians than in Filipinas ($p < 0.001$) (Morton et al., 2003). Contrary to this, a study by Sun et al. (2003), reported no differences in lean mass and fat mass between Asian and Caucasian women, while Alekel et al. (1999), reported a higher lean body mass (kg) (41.5 ± 4.8 vs. 33.7 ± 4.1 ; $p \leq 0.0001$) and lower body fat percentage (%) Caucasians compared to Indian/Pakistani women (30.9 ± 8.0 vs. 38.1 ± 6.9 ; $p \leq 0.0001$).

Table 4. Outcome measures for body composition and muscle strength

Author and year	Outcome for body composition and muscle strength
(Alekel et al., 1999)	Lean body mass (kg) was higher in Caucasians than in Indian/Pakistani women (41.5 ± 4.8 vs. 33.7 ± 4.1 ; $p \leq 0.0001$). Total body fat percent (%) was greater in Indian/Pakistani than in Caucasian women (38.1 ± 6.9 vs. 30.9 ± 8.0 ; $p \leq 0.0001$).
(Davis et al., 1999)	Triceps (10.5 ± 2.7 vs. 9.38 ± 2.12) and Quadriceps muscle (67.8 ± 27.6 vs. 35.7 ± 17.2) strength (kg) was higher in White than in Japanese women ($p < 0.05$).
(Morton et al., 2003)	Total fat mass (23.1 (22.3 – 24.0) vs. 19.8 (18.9 – 20.7) kg) and total lean mass (40.4 (39.9 – 40.9) vs. 36.7 (36.3 – 37.5) kg) was higher in Caucasians than in Filipinas ($p < 0.001$).
(Sun et al., 2003)	Fat mass, body fat percent, lean tissue mass, total appendicular skeletal muscle mass, and leg muscle mass were not different between European- and Asian- Americans.
(Liang et al., 2007)	Total body lean mass and fat mass (kg) was lower in Asians (lean mass: 36.3 ± 3.94 , fat mass: 15.2 ± 20.8) than Caucasians (lean mass: 43.4 ± 6.67 , fat mass: 20.8 ± 7.97 ; $p = 0.01$), and 1 RM leg press strength (lbs) was also lower in Asian (161 ± 39.3) women in comparison to Caucasians (214 ± 46.4).
(McGrath, Ottenbacher, et al., 2017)	Using logistic regression, it was concluded that for every 0.1kg increase in handgrip strength the odds of osteoporosis decrease by 10% in women, and non-Hispanic Asians (odds ratio: 6.42; CI: 6.37-6.48) had a higher odds of osteoporosis in comparison to non-Hispanic whites (odds ratio: 3.97; CI: 3.94-4.00; $p < 0.0001$).

Summary

This review summarized the relationships between bone, muscle, and fat tissues and the differences observed in these tissues in women from Caucasian, East-Asian and South-Asian descents.

Section 1 of the review focusses on the mechanical interactions between bone, muscle, and fat, and discusses the concept of osteo-sarcopenic obesity. This section concludes that muscle mass and strength and bone mineral density are in a linear relationship where an increase in muscle mass increases BMD. However, interactions between bone and fat mass are complex, where an increase in fat mass beyond a certain threshold decreases bone strength. This section also provided an overview of the relationship of vitamin D with BMD and body composition.

Section 2 of the review systematically describes the results of previous literature on racial/ethnic differences in BMD, lean mass, fat mass, and muscle strength in Caucasian, East-Asian and South-Asian women. Bone mineral density is higher or similar in Caucasians than East-Asians. However, only two studies could be traced assessing bone density with DXA in South-Asians. Lean mass and muscle strength were higher in Caucasians in comparison to Asians. Although few studies have illustrated ethnic differences in muscle strength, most of these focus on African-Americans and Caucasians and fail to include a wider range of ethnicities.

Limitations

This review had certain limitations. The research papers used for this review were all in the English language, which restricted the inclusion of non-English language articles. For section 1, the literature was not reviewed systematically and only the articles of interest to the authors were included. Although the authors tried to include all pertinent literature, this may

have resulted in the omission of certain papers that could further elaborate on the relationships between bone, muscle, and fat. For section 2, the literature was reviewed systematically, however, due to the differences in methodological approaches used by different studies, the results were summarized qualitatively, and a meta-analysis of the data was not performed. Although the authors tried to present results controlled for confounding variables, this does not apply to all the studies as few studies did not make adjustments for covariates. The inclusion criteria were stringent and focused only on cross-sectional studies, in healthy, adult women, residing in the United States. Restricting the inclusion criteria to studies including subjects only from the United States severely restrained the number of studies in this review, however, this was necessary to limit the influence of environmental factors on the variables of interest and to at least partially preserve the homogeneity of the studies included in this review.

CHAPTER III

METHODOLOGY

The purpose of this study was to determine group differences and relationships between bone mineral density, bone free lean body mass, fat mass, and muscle strength, in premenopausal women aged 18-45 years belonging to three different racial/ethnic groups: Caucasians, East-Asians (EA), and South-Asians (SA). For each ethnicity, the given age range (18-45 years) was divided into two sub-groups: 18-30 years, and >30 to 45 years, to allow comparison between women who are accruing bone mass vs. those who have achieved their peak bone mass.

Participant Characteristics

One hundred and sixteen recreationally active, premenopausal women aged 18-45 years participated in this study. Recreationally active was defined as being physically active but not following any structured exercise or training regimen for current or future participation in competitive events, such as long-distance running, or weight lifting competitions. Thirteen participants did not return following the initial visit and one participant was perimenopausal, as determined by serum FSH levels, and were therefore excluded from the study, resulting in 102 participants completing the study. The participants were categorized into one of the three independent racial/ethnic groups: Caucasian (Cau; n= 46); East-Asian (EA; n= 29); and South-Asian (SA; n= 27), based on the ethnicity of three out of four of their biological grandparents. On the basis of age, each ethnicity was further sub-divided into two groups: 18-30 years (young (n= 65; Cau= 24, EA= 24, SA= 17)); and >30-45 years (middle-aged (n=38; Cau= 22, EA= 5, SA= 10)). To account for the lower number of middle-aged EA women in the current sample, previous data for five middle-aged EA women was used from a study done in the

Neuromuscular lab in 2016 (IRB No. 6202). This resulted in an increase in total sample size to 107 participants, and to 10 for middle-aged EA women.

The authors were unable to trace any study comparing bone mineral density and its determinants considering East- and South-Asian women as independent groups. Therefore, based on an anticipated statistical power of 0.80, and an effect size of 0.3, a total sample size of 107 was required for the current study. Liang et al. (2007), used a sample size consisting of 36 Caucasians, 39 Hispanics, and 40 Asians, while Misra et al. (2018), included 35 White, 15 Asian-American and 10 Black girls to compare racial/ethnic differences in bone mineral density and its determinants. Based on these studies, the current sample size of 107 (Cau= 46; EA= 29; SA= 27) was sufficient to conduct the primary analyses without the commitment of type II error.

Participants were recruited from Norman, Oklahoma and surrounding areas. Recruitment methods included flyers, word of mouth, emails, message recruitments, and advertisements. All study-related protocols were approved by the Institutional Review Board (IRB) at the University of Oklahoma Health Sciences Center (IRB No. 9314). Participants were screened using a screening checklist questionnaire through email, phone or in person. Participants were excluded from the study if they failed to meet the inclusion criteria based on their responses to the screening checklist questionnaire. Those meeting the inclusion criteria were included in the study and scheduled for their first visit to Neuromuscular lab, in the Department of Health and Exercise Science, University of Oklahoma.

The inclusion and exclusion criteria for the study are as follows:

Inclusion Criteria

1. Participants were healthy, recreationally active, premenopausal women aged 18-45 years.
2. Body weight less than 300 lb (136.3 kg), which is the weight limit for DXA.
3. Height less than 6 feet, to get accurate results on DXA and pQCT.

Exclusion Criteria

1. Women who were pregnant or breastfeeding.
2. Women taking medications that are known to affect bone health eg. mineralo-corticosteroids, glucocorticoids, bisphosphonates, and calcitonin.
3. Women with joint replacements, or any other metal implants in their bodies.
4. Women with recent surgery, fracture, and open wounds.
5. Women who had cardiovascular disease or uncontrolled hypertension.
6. Women who were currently smoking or had smoked regularly within the past 6 months.
7. Women with physical disabilities that can prevent them from performing weight-bearing exercises.

Research Design

This study used a non-randomized cross-sectional study design with three independent racial/ethnic groups: Caucasian, East-Asian, and South-Asian. Participants were categorized into each of these groups based on the ethnicity of three out of four of their biological grandparents. Each of these groups was further divided into two sub-groups based on age: 18-30 years (young group); >30-45 years (middle-age group). This study consisted of three visits, out of which one visit (visit 2) was conducted at the Goddard Health Center at the University of Oklahoma, and the other two visits (visits 1 and 3) were conducted at the Neuromuscular and

Bone Densitometry labs at the Department of Health and Exercise Science, University of Oklahoma.

During the first visit, the participants signed a written and informed consent form and completed the study-related questionnaires. The questionnaires consisted of the ethnicity identification form, health status and menstrual history questionnaires, calcium and vitamin D intake questionnaires, physical activity readiness questionnaire (PAR-Q), bone specific physical activity questionnaire (BPAQ), international physical activity questionnaire (IPAQ), and the sun exposure questionnaire. This was followed by blood pressure measurements and then the participants were familiarized with upper and lower body muscle strength tests which were conducted during visit 3. The participants were encouraged to practice at low intensities to become familiar with the form and technique of the muscle strength tests.

Visit 2 consisted of a fasting morning blood draw (approximately 8 hours fasting; 7.5ml) at the Goddard Health Center, University of Oklahoma, to quantify the serum levels of vitamin D. Serum follicle stimulating hormone levels were assessed in women ≥ 40 years of age to confirm that they were not perimenopausal.

During visit 3, height and weight were measured, and urine samples were assessed for hydration and confirming that the participant was not pregnant. This was followed by the assessment of areal BMD at the total body, lumbar spine and dual proximal femur using Dual Energy X-Ray Absorptiometry and volumetric BMD measurements at 4, 38, and 66% sites for non-dominant tibia using peripheral Quantitative Computed Tomography. Finally, muscle strength tests were performed to assess upper and lower body muscle strength and power, like handgrip test, jump test, and 1RM leg press test.

Procedures

Questionnaires

Ethnicity Identification form: This form was designed to record information regarding the race/ethnicity of the participant; race/ethnicity of the four biological grandparents; country of birth; and the number of years the participant has lived in the United States.

Health Status Questionnaire: This questionnaire was used to check for any health-related issues or medical diagnosis which can limit participation and may impact the results of the study. It consisted of questions related to general health, past illnesses and surgeries, prior medical diagnosis, current and past use of medications, and questions related to usage of tobacco.

Menstrual History Questionnaire: It consisted of questions related to menstrual cycle characteristics, like age of menarche, length of menstrual cycle, irregular or missing periods, and current and past usage of hormonal contraceptives- dosage, type, and duration of use.

Physical Activity Readiness Questionnaire (PAR-Q): This questionnaire was used as a screening tool to check for any physical disabilities which can limit participation in the study (Adams, 1999).

International Physical Activity Questionnaire (IPAQ): This questionnaire consists of questions related to physical activity- job-related; transportation-related; housework, maintenance, caring for the family; recreation and leisure time activities; and time spent sitting. It is based on the activities performed in the past 7 days. Data for each type of activity is converted into metabolic equivalent tasks for seven days (MET-minutes/week) (Hagströmer, Oja, & Sjöström, 2006). It categories individuals into one of the three levels of physical activity- high, moderate, low. High PA category includes 7 days of walking or moderate-intensity activity accumulating at least 3000 MET-minutes/week, or, 3 days of vigorous activity accumulating at least 1500

MET-minutes/week. Moderate PA consists of 3 days of vigorous activity for at least 20 minutes per day, or, 5 days of walking or moderate-intensity activity for at least 30 minutes per day, or, 5 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities achieving at least 600MET-minutes/week. Low PA is the lowest level of activity and consists of those individuals who do not meet the criteria for high or moderate PA levels. IPAQ has been validated with other methods for assessing physical activity scores (Craig et al., 2003).

Bone specific Physical Activity Questionnaire (BPAQ): This questionnaire records bone loading physical activity from 1 year of age to 12 months before testing (past period BPAQ, pBPAQ), and any activity reported from past 12 months (current BPAQ, cBPAQ). This is used to estimate the total BPAQ (tBPAQ) score, which is the calculated average of pBPAQ and cBPAQ scores, using an online BPAQ calculator (Kim, Baker, Sharma-Ghimire, Bembem, & Bembem, 2018). The loading values of BPAQ were determined by measuring ground reaction force for specific bone loading activities (Weeks & Beck, 2008).

Calcium Intake Questionnaire: this questionnaire was used to assess dietary and supplemental calcium intake (mg/day) of the participants over a predetermined period of time. Calcium intake was recorded based on the recall of certain food items and supplements consumed by the participant on a daily or weekly basis. This questionnaire was derived from a validated and quantitative food frequency questionnaire (Musgrave, Giambalvo, Leclerc, Cook, & Rosen, 1989).

Vitamin D intake Questionnaire: This questionnaire recorded the dietary and supplemental intake of vitamin D. This questionnaire was based on recall of certain food items and supplements consumed by the participant on a weekly and monthly basis. To prevent

reporting bias, the amount of vitamin D present in the food items was not revealed to the participant (Taylor, Kruczek, Anderson, Hubbard, & Misra, 2009).

For both calcium and vitamin D supplements, the participants were asked to provide information about the exact amount of calcium (mg) and vitamin D (IU) present in one serving of the supplement or bring their supplements along when they come for the first visit.

Sun Exposure Questionnaire: This questionnaire provides a score out of 56 based on the duration of time spent in the sun and the amount of skin exposed. A higher score is indicative of a longer duration of exposure to sun or more amount of skin exposed or both. This helps to account for the cutaneous synthesis of vitamin D. Along with the sun exposure score, the time of the year during which this questionnaire was completed was also recorded to account for seasonal differences during data analyses (Køster, Søndergaard, Nielsen, Olsen, & Bentzen, 2018).

Anthropometric Measures

Resting brachial systolic and diastolic blood pressures (mmHg) were measured using an automatic blood pressure monitor (Omron, Japan) in a sitting position on the left arm. Two measurements were performed with a time interval of one minute between each measurement. If there was more than 5 mmHg difference, a third measurement was performed and the average of the closest two values was used. If the blood pressure was high ($\geq 140/90$ mmHg), the participant was asked to lie down for 10 minutes following which the measurement was performed again in supine lying. If the blood pressure was still high, the subject's participation was terminated at that point.

Body weight and height were measured without shoes with light clothing using a wall-mounted stadiometer (PAT #290237, Novel Products, Rockton, IL, USA) and a Tanita BWB-800 digital scale (Tanita Corporation of America, Inc., Arlington Heights, IL). Height was

measured to a nearest of 0.5 cm and weight to 0.1 kg. These measures were then used to calculate body mass index (BMI; kg/m²).

Hydration and Pregnancy testing

Prior to the DXA and pQCT scans, the participants provided a urine sample to test for hydration status using an optical refractometer (VEE GEE CLX-1, Rose Scientific Ltd., Alberta, Canada) to ensure that hydration level was within the normal range of 1.004-1.029.

Additionally, pregnancy test strips (SAS Pregnancy Strip, SAS Scientific, San Antonio, TX) were used to confirm that the participant is not pregnant.

Areal Bone Mineral Density

Total body, lumbar spine (L1-L4) and dual proximal femur (total hip, trochanter, femoral neck) areal BMD was assessed using DXA ((DXA, GE Lunar, Prodigy encore software version 10.50.086, Madison, WI, USA). Bone mineral content, BFLBM, fat mass, and percent body fat were also determined from these scans. The DXA was calibrated daily prior to the scans and all the scans were performed by the same investigator.

For the total body measurement participants were required to lie in supine position on the DXA table and their knees and ankles were secured with Velcro straps. For the lumbar spine measurements, a foam block was placed under the participant's feet in order to position the hip at an angle between 45-90 degrees to obtain accurate and high-quality images. The positioning laser was adjusted to approximately 5cm below the umbilicus so that part of L5 and iliac crest, and some part of T12 was visible in the image. For the proximal dual femur scans the participant's feet were positioned in a triangular brace using Velcro straps such that both the left and right femur were internally rotated. The positioning laser was placed in the midline of the thigh and about 4cm inferior to the greater trochanter or 1cm inferior to the pubic symphysis.

The *in vivo* precision and accuracy of the DXA RMS %CV for areal BMD is 0.7% for the total body BMD, 1.4% for the lumbar spine BMD, and 0.6% for total left and right hip, 0.6% for right trochanter, 0.7% for left trochanter, 0.9% for right femoral neck and 1.01% for left femoral neck BMD. The *in vivo* precision of DXA RMS %CV for body composition variables is 2.0% for percent body fat and fat mass, 1.9% for BFLBM, and 1.7% for fat-free mass.

Volumetric Bone Mineral Density

A pQCT XCT 3000 scanner with software version 6.00 (Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to measure total, cortical, and trabecular vBMD (mg/cm^3), BMC, (mg/mm), and area (mm^2) at 4%, 38%, and 66% of non-dominant tibia sites. Compressive, bending and torsional strength were estimated as bone strength (BSI), stress-strain indices (SSI) (mm^3), and moment of inertia (IP). Variables such as periosteal (Peri C) (mm) and endosteal circumference (Endo C) (mm) were indicative of bone size and shape.

The equipment was calibrated before each testing visit using a cone phantom, and after every seven days with a cortical phantom. Prior to the scan, the length of the participants' non-dominant tibia was measured from the tibial plateau to the medial malleolus. This information was entered into the computer and the participant was instructed to position their limb in the gantry and remain still throughout the scan. The scout view was used to acquire the reference point at the medial malleolus. Following this, 3 sets of scans with 15 slices each were performed at 4%, 38%, and 66% of the non-dominant tibia. The *in vivo* precision (RMS %CV) for the tibia ranges from 0.3-1.1% for the total bone variables, 0.7-3% for trabecular bone variables, and 0.2-1.04% for the cortical bone variables.

Muscular Strength Tests

After the DXA and pQCT scans, the muscular strength tests were performed to measure upper and lower body muscle strength and power. These included the handgrip test, vertical jump test, and 1-RM leg press test.

Handgrip Test

Upper body muscle strength was assessed using a Jamar hand-held dynamometer (Takei, Japan). This test was performed with the participant in sitting position, elbow flexed to 90 degrees and forearm in neutral position with wrist between 0 to 30 degrees dorsiflexion and 0 to 15 degrees ulnar deviation. Each hand was tested three times, alternating between the trials, with 60 seconds rest between trials on the same hand. The intraclass correlation (ICC) for the handgrip dynamometer was 0.874.

Vertical Jump Test Measurement

Jump test was performed to measure muscle power and velocity using a jump mat (Just Jump, Probiotic, AL) and a Tendo FiTRODINE power and speed analyzer (Tendo Sports Machines, Trencin, Slovak Republic). The Tendo unit has two parts, 1) a velocity sensor unit, and, 2) a microcomputer. The velocity sensor unit was attached to a standard barbell that was placed closed to the jump mat and connected to the participant's waist by a Velcro strap enabled cable. Body weight of the participant was entered into the microcomputer. The participant performed a counter-movement vertical jump with unrestricted arm motion. A total of three successful jumps were performed with a one-minute rest period between each trial. The jump was considered unsuccessful if the participant tucked the legs or bent the knees mid-air. The procedure for the jump test has been validated in adult populations (Singh et al., 2014).

The Tendo unit determines the average velocity of mass lifted vertically while the microcomputer multiplies the weight of the lifter by acceleration from gravity to estimate the average force in Newton. Average power is the calculated product of average force and average velocity. Airtime and jump height were recorded by the jump mat and handheld computer. The ICC values for jump power, time in air, jump height and velocity, range between 0.80-0.98.

Leg Press Test

Leg muscle strength was determined by a standard 1-Repetition Maximum (1-RM) test. The participants were in a semi-reclined position on a CYBEX two-leg press machine. The participant completed 5-6 repetitions with a load approximately 50% of the body weight. After a one-minute break, the load was increased to 75% of the body weight and the participant was asked to perform 3-4 repetitions. Then following a 2 minutes rest period, loads were increased such that a maximal voluntary effort was reached with 5 more attempts. Each attempt during this part of the test was separated by a 2-4 minutes rest period. The ICC value for leg press was 0.997. The ICC values for the muscle strength tests are taken from previous studies done in the Neuromuscular lab using the same procedures as those used in the current study.

Blood Sampling and Biochemical Analyses

During visit 2, venipuncture blood draws (7.5 ml) were performed for each participant by a registered nurse or phlebotomist in Goddard Health Center at the University of Oklahoma, in the morning after an overnight fast (approximately 8 hours) to measure serum vitamin D and FSH levels.

Blood samples were allowed to clot, centrifuged, and then the serum was transferred to the Bone Density Lab where it was pipetted into 8 micro-tubes. These micro-tubes were then frozen at -84 degrees Celsius until the assays were performed. Commercial Enzyme-Linked

Immunosorbent Assay (ELISA) kits were used to measure vitamin D and FSH levels. All assays were performed following a step-by-step protocol as per the kit manual. Standard precautions were adhered to while handling bodily fluids. Control samples were measured at the beginning and at the end to assess intra-assay precision. Furthermore, the same control samples were also measured in each assay- to measure inter-assay precision. Both intra- and inter-assay coefficient of variation should be less than 10% for good precision.

Vitamin D Assays

Commercial Enzyme-Linked MicroVue Immunoassay Kit (Quidel Corp.) was used to quantitatively assess 25-hydroxy vitamin D₂ and D₃ (25(OH)D) levels in serum. Prior to each assay, the serum samples and all the kit reagents were thawed to room temperature. Thawing was done only once to prevent denaturation of proteins. During this time standards and controls were reconstituted with deionized water and the wash buffer was prepared by adding 1990mL of deionized water to 10mL of wash solution. 50μL of each standard, control, and sample were pipetted in duplicates into the appropriate microplate wells. Following this 150μL of assay buffer was pipetted into the wells using a multichannel pipette. The microplate was then allowed to incubate in dark for 2 hours on a plate shaker at 500rpm. Following this, all procedures for the assay were performed in the dark. During this 2-hour incubation period, the HRP conjugate solution was prepared and allowed to incubate for 1 hour and 45 minutes. After the incubation was complete, the microplate was washed three times using a plate washer by dispensing 0.4mL of wash solution into each well and aspirating the contents of the well. This was followed by addition of 200μL of HRP conjugate solution to each well and then incubation of the microplate for 30 minutes on a plate shaker at 500rpm. The microplate was washed again three times, and TMB substrate was added to each well followed by incubation for 15 minutes

on a plate shaker at 500rpm. Finally, 100 μ L of stop solution was pipetted into each well and the absorbance was read at 450nm. The intra- and inter-assay precision values for this assay were 3.5% and 3.4%, respectively.

Follicle Stimulating Hormone Assay

Commercial Enzyme-Linked DRG Immunoassay Kit (DRG, Germany) was used to quantitatively assess follicle stimulating hormone (FSH) levels in serum. Prior to each assay, the serum samples and all the kit reagents were thawed to room temperature. Thawing was done only once to prevent denaturation of proteins. During this time standards and controls were reconstituted with 1mL deionized water. The serum samples and kit reagents were allowed to reach room temperature. 25 μ L of each standards, controls, and samples were added to each well in duplicates. This was followed by addition of 100 μ L of enzyme conjugate and mixing of the contents of the plate. The plate was then incubated for 30 minutes and washed 5 times with deionized water, followed by addition of 100 μ L of substrate solution. The plate was again incubated for 10 minutes followed by addition 50 μ L of stop solution. Finally, the absorbance was read at 450nm. The intra-assay precision for this assay was 4.4%.

Data Analyses

Data were analyzed using SPSS (SPSS Inc., Chicago, IL, version 24.0). Descriptive statistics were reported as mean \pm SE. All dependent variables (areal and volumetric BMD; body composition; muscle strength parameters) were tested for normality using the Kolmogorov-Smirnov test. Two-way ANCOVA (Ethnicity X Age) was used to determine the main effects and interactions of ethnicity and age on the dependent variables, followed by Bonferroni post-hoc analysis. To control for the influence of body size on bone density, body composition, and muscle strength variables, height and weight were used as covariates. Previous studies have

reported that bone density and lifestyle factors vary between foreign-born and U.S. born individuals belonging to the same ethnicity (Davis, Nevitt, Wasnich, & Ross, 1999). Therefore, in order to control for these variations, the duration of time the participant has lived in the U.S. was also used as a covariate in addition to height and weight. Additionally, Zero-order Pearson correlation coefficients were used to determine relationships between the dependent variables separately for the three ethnic groups. Chi-square analyses were conducted between ethnicity, and other categorical variables to determine association and sampling distribution. Finally, multiple linear regression analysis was conducted to examine the association between dependent and independent variables. The effect sizes were represented as partial eta-squared values and classified as small (0.0099), medium (0.0588) and large (0.1379) (Richardson, 2011). The level of significance was set at $p < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to determine group differences and relationships between bone mineral density, bone free lean body mass, fat mass, and muscle strength, in young and middle-aged premenopausal women aged 18-45 years belonging to three different racial/ethnic groups: Caucasians (Cau), East-Asians (EA), South-Asians (SA). For each ethnicity, the given age range (18-45 years) was further divided into two sub-groups: 18-30 years, and >30 to 45 years, to allow comparison between women who are accruing bone mass vs. those who have achieved their peak bone mass. This study also evaluated differences in physical activity levels and serum concentration of vitamin D between the three racial/ethnic groups across the two age groups.

Participants

A total of 116 recreationally active premenopausal women participated in this cross-sectional study. This study included a total of three visits. Thirteen participants did not complete all the three visits, and one woman was perimenopausal based on FSH levels and was therefore excluded from the study. This resulted in 102 participants completing the entire study. These participants were categorized into one of the three independent racial/ethnic groups: Caucasian (n=46), East-Asian (n=29), and South-Asian (n=27), based on the ethnicity of three out of four of their biological grandparents. Based on age, these groups were further subdivided into young (18-30 years: n= 65; Cau= 24, EA= 24, SA= 17) and middle-aged (>30-45 years: n=38; Cau= 22, EA= 5, SA= 10). Taking into consideration the small sample size for middle-aged East-Asian women, data for five middle-aged East-Asian (Chinese) women were used from a previous study done in the Neuromuscular lab (IRB # 6202). This increased the

number of middle-aged East-Asian women to 10 participants and the total sample size to 107 participants for the current study.

Table 5 describes the participant characteristics including their age, height, weight, age of menarche, and duration of stay in the U.S. for the three ethnicities across the two age groups. A two-way ANOVA was used to determine the main effects and interactions of ethnicity and age. Significant main effects of ethnicity and age were observed; however, no Ethnicity X Age interaction was noted.

There were significant main effects of ethnicity for age, height, weight, and duration of stay in the United States. Caucasian and South-Asian women were older (Cau: 29.73 ± 1.05 , SA: 29.80 ± 1.69 vs. EA: 25.67 ± 1.15 years; $p=0.001$; $\eta_p^2=0.732$) and weighed more in comparison to East-Asian women (Cau: 70.32 ± 2.68 , SA: 70.46 ± 2.84 vs. EA: 57.62 ± 1.84 kg; $p=0.01$; $\eta_p^2=0.086$). Caucasians were also significantly taller (Cau: 165.82 ± 0.92 vs. SA: 160.36 ± 1.28 , EA: 159.47 ± 0.90 cm; $p=0.001$; $\eta_p^2=0.174$) and had longer duration of stay in U.S. (Cau: 28.64 ± 1.32 vs. SA: 14.73 ± 2.11 , EA: 16.53 ± 1.75 years; $p=0.001$; $\eta_p^2=0.338$) than South-Asian and East-Asian women.

On the basis of age, middle aged women were older (Middle-aged: 36.59 ± 0.63 vs. Young: 23.22 ± 0.49 years; $p=0.001$; $\eta_p^2=0.174$), weighed more (Middle-aged: 70.11 ± 2.48 vs. Young: 63.13 ± 1.88 kg; $p=0.03$; $\eta_p^2=0.48$) and had longer duration of stay in United States (Middle-aged: 25.82 ± 1.34 vs. Young: 16.07 ± 1.06 years; $p=0.001$; $\eta_p^2=0.237$) than younger women. There were no significant main effects of age ($p=0.75$) or ethnicity ($p=0.51$) for age of menarche between the participants.

Additionally, chi-square analyses were used to determine the association between ethnicity and oral contraceptive use (Table 6) and country of birth of the participants (Table 7).

The association between the variables was significant both for oral contraceptive use ($p=0.001$) and country of birth ($p=0.001$). The total number of East-Asian women in this study were 34, which includes the data for 5 women which was used from a previous study (IRB # 6202). We did not have any record for oral contraceptive use for these women and hence table 6 reports the data for 29 East-Asian women who were recruited as a part of the current study. A total of 27.2% (28/103) participants were currently consuming oral contraceptives, from which 82.1% (23/28) were Caucasians, 14.3% (4/28) were East-Asians, while only 3.6% (1/28) were South-Asians. Additionally, 60.7% (65/107) of the total participants were born in the United States, out of which 67.7% (44/65) were Caucasians, 21.5% (14/65) were East-Asians and 10.8% (7/65) were South-Asians.

Table 6. Frequency of distribution for current OC use between Caucasian, East-Asian, and South-Asian women (%)

		Ethnicity				
		Caucasian	East-Asian	South-Asian	Total	
Use of OC**	OC Non-Users	Count	23	25	27	75
		% within use of OC	30.7%	33.3%	36.0%	100.0%
		% within Ethnicity	50.0%	86.2%	96.4%	72.8%
		% of Total	22.3%	24.3%	26.2%	72.8%
	OC Users	Count	23	4	1	28
		% within use of OC	82.1%	14.3%	3.6%	100.0%
		% within Ethnicity	50.0%	13.8%	3.6%	27.2%
		% of Total	22.3%	3.9%	1.0%	27.2%
	Total	Count	46	29	28	103
		% within use of OC	44.7%	28.2%	27.2%	100.0%
% within Ethnicity		100.0%	100.0%	100.0%	100.0%	
% of Total		44.7%	28.2%	27.2%	100.0%	

**p<0.01, Significant chi-square statistic. OC, oral contraceptive.

Table 7. Frequency of distribution for country of birth between Caucasian, East-Asian, and South-Asian women (%)

		Ethnicity				
		Caucasian	East-Asian	South-Asian	Total	
Country of Birth**	Foreign Born	Count	2	20	20	42
		% within Country of Birth	4.8%	47.6%	47.6%	100.0%
		% within Ethnicity	4.3%	58.8%	74.1%	39.3%
		% of Total	1.9%	18.7%	18.7%	39.3%
	U.S. Born	Count	44	14	7	65
		% within Country of Birth	67.7%	21.5%	10.8%	100.0%
		% within Ethnicity	95.7%	41.2%	25.9%	60.7%
		% of Total	41.1%	13.1%	6.5%	60.7%
	Total	Count	46	34	27	107
		% within Country of Birth	43.0%	31.8%	25.2%	100.0%
% within Ethnicity		100.0%	100.0%	100.0%	100.0%	
% of Total		43.0%	31.8%	25.2%	100.0%	

**p<0.01, Significant chi-square statistic. U.S., United States.

Physical Activity, Calcium and Vitamin D Intakes

Table 8 summarizes the results for physical activity scores, calcium and vitamin D intakes, serum vitamin D levels and sun exposure scores for participants in the three racial/ethnic groups across the two age groups. A two-way ANCOVA was used to determine the main effects and interactions of ethnicity and age by using the duration of stay in U.S. as a covariate. There were no significant main effects of age or Ethnicity X Age interactions before or after adjusting for the covariate. However, significant main effects of ethnicity were noted both before and after adjustment for the covariate.

Prior to controlling for covariate, past (Cau: 60.62 ± 7.79 vs. EA: 30.51 ± 9.31 , SA: 22.76 ± 5.25 ; $p=0.004$; $\eta_p^2=0.103$) and total (Cau: 33.13 ± 4.17 vs. EA: 17.13 ± 4.94 , SA: 13.67 ± 2.67 ; $p=0.005$; $\eta_p^2=0.098$) BPAQ scores were significantly greater in Caucasians in comparison to East-Asian and South Asian women. Although no significant main effects or interactions were observed for daily vitamin D intake, sun exposure scores were significantly higher in Caucasian and East-Asian women in comparison to South-Asians (Cau: 18.26 ± 1.54 , EA: 21.90 ± 1.98 vs. SA: 12.21 ± 1.41 ; $p=0.001$; $\eta_p^2=0.136$). Moreover, daily calcium intake (mg/day) was higher in Caucasians and South-Asians compared to East-Asians (Cau: 893.07 ± 52.95 , SA: 964.21 ± 110.20 vs. EA: 608.15 ± 52.65 ; $p=0.002$; $\eta_p^2=0.116$). Serum vitamin D levels (ng/mL) were significantly greater in Caucasians compared to East- and South-Asian women (Cau: 32.50 ± 2.85 vs. EA: 24.80 ± 1.62 , SA: 23.48 ± 2.21 ; $p=0.04$; $\eta_p^2=0.061$). No significant differences were observed for physical activity measured using IPAQ in Mets/min, and cBPAQ scores across the three racial/ethnic groups.

After adjusting these results for duration of stay in U.S. (Table 8), the differences remained significant for calcium intake ($p=0.001$; $\eta_p^2=0.127$), serum vitamin D levels ($p=0.01$;

$\eta_p^2=0.084$) and sun exposure scores ($p=0.001$; $\eta_p^2=0.133$). Daily calcium intake was higher in Caucasians ($p=0.003$) and South-Asians ($p=0.008$) than in East-Asians, and, sun exposure scores were higher in Caucasians and East-Asians compared to South-Asians ($p=0.001$). Serum vitamin D levels were higher in Caucasians compared to both East- ($p=0.04$) and South-Asians ($p=0.02$). Following adjustment for duration of stay in U.S., the past ($p=0.05$) and total ($p=0.06$) BPAQ scores became similar across the three racial/ethnic groups.

Tables 9 and 10 describe the chi-square analyses showing sampling distributions between ethnicity and physical activity and serum vitamin D levels. The International Physical Activity Questionnaire (IPAQ) was used to determine physical activity levels. The participants were classified as having low, moderate, or high physical activity levels based on the criterion described in detail in the ‘Methodology’ section under ‘Procedures’. The chi-square test between ethnicity and PA levels was not significant ($p=0.16$), however, out of the total 107 participants, 31.8% (34/107) had high levels of physical activity, while 57.9% (62/107) and 10.3% (11/107) had moderate and low levels of physical activity respectively. For vitamin D, individuals with serum vitamin D levels (25(OH)D) ≥ 30 ng/mL were classified as having sufficient vitamin D, while those with levels between 21-29ng/mL and below 20ng/mL were classified as insufficient and deficient respectively (Holick, 2009; Lee, Gadi, Spertus, Tang, & O’Keefe, 2011). From the entire sample, 30.8% (33/107) of the participants had sufficient vitamin D levels, out of which 67.7% (22/33) were Caucasians, 18.2% (6/33) were East-Asians and 15.2% (5/33) were South-Asians. Moreover, 32.7% (35/107) had insufficient vitamin D levels, consisting of 40.0% (14/35) Caucasians, 45.7% (16/35) East-Asians, and 14.3% (5/35) South-Asians, while 36.4% (39/107) had deficient vitamin D levels, including 25.6% (10/39) Caucasians, 30.8% (12/39) East-Asians, and 43.6% (17/39) South-Asians.

Table 8. Physical activity and calcium and vitamin D intake for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean ± SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Total PA score (mets/min)	3044.46 ± 417.35	2445.35 ± 272.69	1873.04 ± 298.8	1811.00 ± 418.9	2315.21 ± 330.12	2890.64 ± 607.4
cBPAQ score	7.53 ± 2.10	3.58 ± 1.25	3.67 ± 1.17	3.90 ± 2.37	2.94 ± 0.74	7.54 ± 3.32
pBPAQ score	70.11 ± 12.53	50.27 ± 8.65	29.13 ± 10.00	33.84 ± 21.56	19.54 ± 6.49	29.97 ± 9.64
tBPAQ score	38.82 ± 6.74	26.93 ± 4.49	16.40 ± 5.16	18.87 ± 11.87	11.24 ± 3.36	18.76 ± 4.32
Calcium Intake (mg/day) ^{††}	857.23 ± 80.59 ^{ββ}	932.18 ± 68.27 ^{ββ}	648.44 ± 57.2	511.46 ± 114.2	1018.36 ± 173.6 ^{ββ}	851.53 ± 91.7 ^{ββ}
Vitamin D Intake (IU/day)	398.71 ± 107.85	1302.26 ± 313.98	343.19 ± 96.18	817.33 ± 199.66	1196.38 ± 442.30	629.23 ± 252.47
Sun Exposure Score ^{††}	22.58 ± 1.93 ^{γγ}	13.55 ± 2.04 ^{γγ}	21.50 ± 2.29 ^{γγ}	23.80 ± 3.65 ^{γγ}	14.24 ± 1.99	9.09 ± 1.52
Serum Vitamin D levels (ng/mL) ^{††}	36.04 ± 4.58 ^{β,γ}	28.63 ± 3.18 ^{β,γ}	24.29 ± 2.01	26.04 ± 2.85	21.69 ± 2.68	26.53 ± 3.68

*p<0.05, **p<0.01, Significant age difference (Young vs. Middle-aged); †p<0.05, ††p<0.01, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian; p<0.01, ^{αα} Compared to Caucasians, ^{ββ} Compared to East-Asian, ^{γγ} Compared to South-Asian); ^δ p<0.05, ^{δδ} p<0.01 Significant Ethnicity X Age interaction. BPAQ, Bone-Specific Physical Activity; cBPAQ, current BPAQ; pBPAQ, past BPAQ; tBPAQ, total BPAQ.

Table 9. Frequency of distribution for PA levels between Caucasian, East-Asian, and South-Asian women (%)

		Ethnicity				
		Caucasian	East-Asian	South-Asian	Total	
PA Level	Low	Count	4	6	1	11
		% within PA level	36.4%	54.5%	9.1%	100.0%
		% within Ethnicity	8.7%	17.6%	3.7%	10.3%
		% of Total	3.7%	5.6%	0.9%	10.3%
	Moderate	Count	25	22	15	62
		% within PA level	40.3%	35.5%	24.2%	100.0%
		% within Ethnicity	54.3%	64.7%	55.6%	57.9%
		% of Total	23.4%	20.6%	14.0%	57.9%
	High	Count	17	6	11	34
		% within PA level	50.0%	17.6%	32.4%	100.0%
		% within Ethnicity	37.0%	17.6%	40.7%	31.8%
		% of Total	15.9%	5.6%	10.3%	31.8%
Total	Count	46	34	27	107	
	% within PA level	43.0%	31.8%	25.2%	100.0%	
	% within Ethnicity	100.0%	100.0%	100.0%	100.0%	
	% of Total	43.0%	31.8%	25.2%	100.0%	

PA, physical activity

Table 10. Frequency of distribution for serum vitamin D levels between Caucasian, East-Asian, and South-Asian women (%)

		Ethnicity				
		Caucasian	East-Asian	South-Asian	Total	
Vitamin D Level**	Deficient	Count	10	12	17	39
		% within vitamin D level	25.6%	30.8%	43.6%	100.0%
		% within Ethnicity	21.7%	35.3%	63.0%	36.4%
		% of Total	9.3%	11.2%	15.9%	36.4%
	Insufficient	Count	14	16	5	35
		% within vitamin D level	40.0%	45.7%	14.3%	100.0%
		% within Ethnicity	30.4%	47.1%	18.5%	32.7%
		% of Total	13.1%	15.0%	4.7%	32.7%
	Sufficient	Count	22	6	5	33
		% within vitamin D level	66.7%	18.2%	15.2%	100.0%
		% within Ethnicity	47.8%	17.6%	18.5%	30.8%
		% of Total	20.6%	5.6%	4.7%	30.8%
Total	Count	46	34	27	107	
	% within vitamin D level	43.0%	31.8%	25.2%	100.0%	
	% within Ethnicity	100.0%	100.0%	100.0%	100.0%	
	% of Total	43.0%	31.8%	25.2%	100.0%	

**p<0.01, Significant chi-square statistic.

Body Composition

Table 11 depicts the main effects and interactions of ethnicity and age for body composition variables for young and middle-aged Caucasian, East- and South-Asian women, after controlling for height, weight, and duration of stay in the U.S. There were main effects of ethnicity and age, but no significant Ethnicity X Age interactions before or after adjustment for covariates.

There were significant main effects of ethnicity for total body fat percentage, fat mass, estimated visceral adipose tissue (VAT) mass, BFLBM, and appendicular skeletal muscle mass (ASM), and of age for fat mass, A/G ratio and estimated VAT mass before adjustment for covariates. Based on ethnicity, total body fat percentage (%) was higher in South-Asians in comparison to East-Asian and Caucasian women (SA: 41.05 ± 0.98 vs. EA: 32.74 ± 0.98 , Cau: 34.36 ± 1.13 ; $p=0.001$; $\eta_p^2=0.220$), whereas fat mass (kg) was higher for both South-Asian and Caucasian women compared to East-Asians (SA: 29.40 ± 1.75 , Cau: 25.02 ± 1.74 vs. EA: 19.07 ± 1.13 ; $p=0.001$; $\eta_p^2=0.150$). South-Asians also had higher estimated VAT mass (g) than Caucasians (SA: 576.68 ± 79.39 vs. Cau: 408.74 ± 80.15 ; $p=0.03$; $\eta_p^2=0.060$). Bone free lean body mass (Cau: 42.27 ± 1.16 vs. EA: 36.31 ± 0.87 kg; $p=0.001$; $\eta_p^2=0.120$) and ASM (Cau: 19.27 ± 0.56 vs. EA: 19.27 ± 0.56 kg; $p=0.001$; $\eta_p^2=0.157$) were significantly higher in Caucasians compared to East-Asian women.

Additionally, significant main effects of age were observed for fat mass (Middle-aged: 27.01 ± 1.54 vs. Young: 22.55 ± 1.19 kg; $p=0.02$; $\eta_p^2=0.050$), A/G ratio (Middle-aged: 0.43 ± 0.02 vs. Young: 0.35 ± 0.02 ; $p=0.003$; $\eta_p^2=0.080$), and estimated VAT mass (Middle-aged: 577.67 ± 69.83 vs. Young: 317.69 ± 54.15 g; $p=0.004$; $\eta_p^2=0.080$), where middle aged women had higher values for each of these variables in comparison to younger women.

Following adjustment for covariates, no significant main effects of age were observed. For ethnicity, the differences remained significant only for total body fat percentage (BF%) and fat mass and became significant for A/G ratio (Table 11). Total body fat percentage ($p=0.008$; $\eta_p^2=0.092$) and fat mass ($p=0.02$; $\eta_p^2=0.078$) were significantly greater in South-Asian women than East-Asians (SA vs. EA: BF%, $p= 0.01$; Fat mass, $p= 0.03$) and Caucasians (SA vs. Cau: BF%, $p=0.04$; Fat mass, $p=0.04$). Moreover, A/G ratio was significantly higher ($p=0.003$; $\eta_p^2=0.110$) in East-Asians compared to Caucasian women ($p=0.002$).

Table 11. Body composition variables for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean \pm SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Body Fat % ^{††}	32.32 \pm 1.41	36.59 \pm 1.70	32.63 \pm 1.35	33.00 \pm 0.90	39.76 \pm 1.38 ^{$\alpha,\beta\beta$}	43.15 \pm 1.12 ^{$\alpha,\beta\beta$}
Fat mass (kg) [†]	21.55 \pm 2.08	28.80 \pm 2.66	18.95 \pm 1.54	19.35 \pm 1.19	27.15 \pm 2.20 ^{α,β}	33.54 \pm 2.85 ^{α,β}
A/G Ratio ^{††}	0.32 \pm 0.03	0.40 \pm 0.03	0.35 \pm 0.03 ^{$\alpha\alpha$}	0.41 \pm 0.03 ^{$\alpha\alpha$}	0.33 \pm 0.03	0.50 \pm 0.04
eVAT mass (g)	254.00 \pm 77.47	577.55 \pm 137.8	271.67 \pm 71.13	348.10 \pm 52.66	427.41 \pm 82.30	807.36 \pm 133.4
BFLBM (kg)	40.41 \pm 1.73	44.30 \pm 1.44	36.05 \pm 1.04	36.94 \pm 1.66	37.44 \pm 1.42	41.26 \pm 2.18
ASM (kg)	18.70 \pm 0.83	19.89 \pm 0.72	15.64 \pm 0.54	15.92 \pm 0.92	17.29 \pm 0.73	18.56 \pm 1.17

[†]p<0.05, ^{††}p<0.01, Significant ethnicity difference (p<0.05, ^{α} Compared to Caucasians, ^{β} Compared to East-Asian, ^{γ} Compared to South-Asian; p<0.01, ^{$\alpha\alpha$} Compared to Caucasians, ^{$\beta\beta$} Compared to East-Asian, ^{$\gamma\gamma$} Compared to South-Asian). A/G, Android/Gynoid; eVAT, estimated visceral adipose tissue; BFLBM, bone free lean body mass; ASM, appendicular skeletal muscle mass.

Total Body, Lumbar Spine, and Dual Proximal Femur Areal Bone Mineral Density

Table 12 shows the results for total body and lumbar spine (LS) areal BMD, Z-scores, and BMC across ethnicity and age following adjustment for height, weight, and duration of stay in the U.S. There were no significant interactions of Ethnicity X Age before or after controlling for covariates.

Prior to adjustment for covariates, there were significant main effects of ethnicity and age for BMC at the total body and lumbar spine. For ethnicity, total body BMC (g) was significantly higher in Caucasians compared to East- and South-Asian women (Cau: 2385.428 ± 57.695 vs. EA: 2126.432 ± 42.203 , SA: 2170.543 ± 46.166 ; $p=0.001$; $\eta_p^2=0.125$), while LS BMC was higher in Caucasians in comparison to East-Asian women (Cau: 66.489 ± 1.540 vs. EA: 59.076 ± 1.480 ; $p=0.006$; $\eta_p^2=0.032$). On the basis of age, both total body (Middle-aged: 2303.294 ± 49.973 vs. Young: 2168.996 ± 38.755 g; $p=0.04$; $\eta_p^2=0.042$) and LS BMC (Middle-aged: 64.510 ± 1.591 vs. Young: 60.119 ± 1.234 g; $p=0.04$; $\eta_p^2=0.045$) were higher in middle-aged compared to younger women. Similarly, total body (Middle-aged: 1.197 ± 0.02 vs. Young: 1.111 ± 0.017 g/cm²; $p=0.003$; $\eta_p^2=0.086$) and LS BMD (Middle-aged: 1.241 ± 0.018 vs. Young: 1.191 ± 0.014 g/cm²; $p=0.03$; $\eta_p^2=0.045$) was also higher in middle-aged juxtaposed to younger women. However, following adjustments for covariates, these differences were minimized, and no significant main effects of ethnicity or age were noted (Table 12).

Table 13 depicts the results for areal BMD and BMC at the femoral neck (FN), trochanter, and total hip (TH) for both left and right sides across ethnicity and age following adjustment for height, weight, and duration of stay in U.S. There were significant main effects of ethnicity and Ethnicity X Age interactions, however, there were no main effects of age before

or after controlling for covariates. Moreover, none of the participants had Z-score values less than -2 for femoral neck BMD.

Before controlling for covariates, Caucasians had a higher FN BMD for both right (Cau: 1.030 ± 0.016 vs. EA: 0.956 ± 0.019 g/cm²; $p=0.003$; $\eta_p^2=0.107$) and left sides in comparison to East-Asians, and higher for only left side compared to South-Asian women (Cau: 1.035 ± 0.015 vs. EA: 0.950 ± 0.018 , SA: 0.996 ± 0.024 g/cm²; $p=0.001$; $\eta_p^2=0.140$). Bone mineral contact at left (Cau: 4.825 ± 0.090 vs. EA: 4.335 ± 0.088 g; $p=0.001$; $\eta_p^2=0.145$) and right (Cau: 4.828 ± 0.099 vs. EA: 4.349 ± 0.082 g; $p=0.005$; $\eta_p^2=0.111$) femur neck was higher in Caucasian than in East-Asians. Bone mineral density and BMC at left (BMD, Cau: 0.825 ± 0.015 vs. EA: 0.759 ± 0.015 g/cm²; $p=0.003$; $\eta_p^2=0.105$; BMC, Cau: 9.428 ± 0.307 vs. EA: 7.455 ± 0.242 g; $p=0.001$; $\eta_p^2=0.180$) and right (BMD, Cau: 0.822 ± 0.015 vs. EA: 0.767 ± 0.016 g/cm²; $p=0.02$; $\eta_p^2=0.076$; BMC, Cau: 9.508 ± 0.320 vs. EA: 7.604 ± 0.250 g; $p=0.001$; $\eta_p^2=0.151$) trochanter was also higher in Caucasian than in East-Asian women. Similarly, left total hip BMD (Cau: 1.038 ± 0.016 vs. EA: 0.983 ± 0.017 g/cm²; $p=0.03$; $\eta_p^2=0.066$) and total hip BMC at both left (Cau: 31.313 ± 0.694 vs. EA: 27.520 ± 0.662 g; $p=0.001$; $\eta_p^2=0.145$) and right (Cau: 31.304 ± 0.704 vs. EA: 27.806 ± 0.651 ; $p=0.001$ g; $\eta_p^2=0.121$) sides was higher in Caucasians compared to East-Asian women.

After controlling for covariates (Table 13), the differences remained significant only at the left femoral neck for areal BMD ($p=0.01$; $\eta_p^2=0.085$) with Caucasians having higher values in comparison to East-Asian women ($p=0.01$). Significant Ethnicity X Age interaction was observed for left FN BMD ($p=0.04$; $\eta_p^2=0.061$). Younger Caucasian women had a higher left FN BMD than younger South-Asian women ($p=0.04$), whereas the BMD values were lower for

middle-aged East-Asian women in comparison to both middle-aged Caucasian ($p=0.008$) and South-Asian ($p=0.04$) women.

Table 12. Total body and lumbar spine areal bone mineral density for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean ± SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Total body aBMD (g/cm ²)	1.144 ± 0.025	1.254 ± 0.024	1.117 ± 0.019	1.125 ± 0.033	1.073 ± 0.055	1.212 ± 0.034
Total body BMC (g)	2267.32 ± 83.5	2514.27 ± 71.1	2114.65 ± 47.8	2154.70 ± 89.5	2125.01 ± 52.1	2240.91 ± 84.4
Total body Z-scores	0.974 ± 0.121	1.300 ± 0.180	1.016 ± 0.149	0.680 ± 0.272	0.593 ± 0.142	0.964 ± 0.256
Spine L1-L4 aBMD (g/cm ²)	1.222 ± 0.021	1.261 ± 0.025	1.169 ± 0.021	1.193 ± 0.043	1.180 ± 0.024	1.271 ± 0.046
Spine L1-L4 BMC (g)	65.00 ± 2.15	68.11 ± 2.21	58.59 ± 1.86	60.23 ± 2.41	56.76 ± 2.04	65.18 ± 3.65
Spine L1-L4 Z-scores	0.447 ± 0.160	0.345 ± 0.171	0.163 ± 0.191	0.330 ± 0.299	-0.127 ± 0.221	0.509 ± 0.359

*p<0.05, **p<0.01, Significant age difference (Young vs. Middle-aged); †p<0.05, ††p<0.01, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian; p<0.01, ^{αα} Compared to Caucasians, ^{ββ} Compared to East-Asian, ^{γγ} Compared to South-Asian); ^δ p<0.05, ^{δδ} p<0.01 Significant Ethnicity X Age interaction. aBMD, areal bone mineral density; L1-L4, lumbar vertebrae 1 to 4.

Table 13. Hip areal BMD for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean ± SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Right FN aBMD (g/cm ²)	1.031 ± 0.019	1.029 ± 0.026	0.983 ± 0.022	0.892 ± 0.030	0.971 ± 0.029	1.049 ± 0.039
Left FN aBMD (g/cm ²) ^{†δ}	1.036 ± 0.018 ^β	1.033 ± 0.024 ^β	0.977 ± 0.020	0.887 ± 0.031	0.965 ± 0.027	1.045 ± 0.041
Right FN Z-scores	0.005 ± 0.114	0.050 ± 0.153	-0.432 ± 0.161	-0.650 ± 0.201	-0.467 ± 0.206	0.282 ± 0.237
Left FN Z-scores [†]	0.089 ± 0.105 ^β	0.064 ± 0.145 ^β	-0.458 ± 0.153	-0.680 ± 0.208	-0.553 ± 0.185	0.255 ± 0.245
Right FN BMC (g)	4.721 ± 0.124	4.945 ± 0.156	4.442 ± 0.095	4.126 ± 0.143	4.379 ± 0.126	4.727 ± 0.208
Left FN BMC (g)	4.756 ± 0.115	4.899 ± 0.141	4.418 ± 0.099	4.137 ± 0.171	4.344 ± 0.116	4.726 ± 0.185
Right Troch aBMD (g/cm ²)	0.815 ± 0.018	0.830 ± 0.024	0.787 ± 0.019	0.720 ± 0.028	0.770 ± 0.023	0.818 ± 0.037
Left Troch aBMD (g/cm ²)	0.815 ± 0.018	0.836 ± 0.024	0.781 ± 0.017	0.706 ± 0.027	0.759 ± 0.025	0.815 ± 0.039
Right Troch Z-scores	-0.316 ± 0.144	-0.259 ± 0.169	-0.511 ± 0.161	-0.820 ± 0.228	-0.753 ± 0.173	-0.260 ± 0.325
Left Troch Z-scores	-0.274 ± 0.133	-0.209 ± 0.162	-0.511 ± 0.149	-0.920 ± 0.209	-0.893 ± 0.171	-0.250 ± 0.332
Right Troch BMC (g)	9.213 ± 0.429	9.830 ± 0.480	7.566 ± 0.318	7.693 ± 0.395	8.099 ± 0.350	8.686 ± 0.738
Left Troch BMC (g)	9.014 ± 0.426	9.880 ± 0.433	7.502 ± 0.307	7.342 ± 0.387	8.109 ± 0.377	8.943 ± 0.580
Right THIP aBMD (g/cm ²)	1.028 ± 0.019	1.047 ± 0.027	1.016 ± 0.020	0.935 ± 0.035	0.992 ± 0.031	1.043 ± 0.040
Left THIP aBMD (g/cm ²)	1.031 ± 0.019	1.045 ± 0.026	1.006 ± 0.019	0.928 ± 0.034	0.980 ± 0.030	1.033 ± 0.042
Right THIP Z-scores	0.179 ± 0.114	0.236 ± 0.164	0.142 ± 0.173	-0.290 ± 0.290	-0.153 ± 0.237	0.290 ± 0.313
Left THIP Z-scores	0.237 ± 0.126	0.218 ± 0.167	0.068 ± 0.170	-0.330 ± 0.239	-0.300 ± 0.223	0.220 ± 0.326
Right THIP BMC (g)	30.328 ± 0.907	32.368 ± 1.064	28.079 ± 0.777	27.150 ± 1.233	28.330 ± 0.858	29.831 ± 1.408
Left THIP BMC (g)	30.452 ± 0.960	32.253 ± 0.988	27.887 ± 0.786	26.638 ± 1.246	28.025 ± 0.831	29.887 ± 1.269

[†]p<0.05, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian); ^δ p<0.05, Significant Ethnicity X Age interaction. aBMD, areal bone mineral density; BMC, bone mineral content; FN, femur neck; Troch, trochanter, THIP, total hip.

Volumetric Bone Mineral Density at 4, 38, and 66% of Non-Dominant Tibia

Table 14 describes the results for pQCT measured volumetric BMD variables and estimated strength indices at 4% of non-dominant tibia following adjustment for covariates, height, weight, and duration of stay in the U.S. There were no significant main effects of age before or after controlling for covariates.

Before controlling for covariates, significant main effects of ethnicity were observed for total area and periosteal circumference, and a significant interaction was noted for trabecular vBMD. Both total area (Cau: 981.224 ± 18.135 vs. SA: 903.646 ± 28.321 mm²; $p=0.04$; $\eta_p^2=0.064$) and periosteal circumference (Cau: 110.830 ± 1.024 vs. SA: 106.204 ± 1.681 mm; $p=0.03$; $\eta_p^2=0.067$) were higher in Caucasian compared to South-Asian women. Trabecular vBMD was higher ($p=0.008$; $\eta_p^2=0.091$) in younger East-Asian women in comparison to younger South-Asian women ($p=0.001$), and within East-Asians, it was higher in younger than in middle-aged women ($p=0.01$).

Following adjustment for covariates, the differences were minimized and remained significant only for trabecular vBMD ($p=0.03$; $\eta_p^2=0.067$) which was higher in East-Asians compared to South-Asians for younger women ($p=0.01$), and higher for young than middle-aged within East-Asians ($p=0.04$) (Table 14).

Table 15 depicts the results for pQCT measured variables at 38% of the non-dominant tibia which have been controlled for covariates of height, weight, and duration of stay in the U.S. There were no significant interactions or main effects of age before or after controlling for covariates.

Prior to adjustment for covariates, significant main effects of ethnicity were noted for total BMC and vBMD, cortical BMC and area, and SSI. Both total BMC (Cau: 337.538 ± 7.004

vs. EA: 304.176 ± 8.810 , SA: 297.043 ± 6.612 mg/mm; $p=0.001$; $\eta_p^2=0.123$) and vBMD (Cau: 951.646 ± 6.021 vs. EA: 919.209 ± 11.504 , SA: 907.350 ± 11.181 mg/cm³; $p=0.002$; $\eta_p^2=0.117$) were significantly higher in Caucasians in comparison to East- and South-Asian women. Similarly, cortical BMC (Cau: 323.295 ± 6.774 vs. EA: 292.317 ± 8.728 , SA: 284.963 ± 6.276 mg/mm; $p=0.002$; $\eta_p^2=0.116$) and area (Cau: 269.701 ± 5.716 vs. EA: 243.544 ± 7.361 , SA: 238.760 ± 5.710 mm²; $p=0.003$; $\eta_p^2=0.108$) were also higher in Caucasians compared to East- and South-Asians. Moreover, Caucasians had higher values for stress-strain index (SSI) in contrast to South-Asian women (Cau: 1505.283 ± 43.866 vs. SA: 1302.899 ± 45.113 mm³; $p=0.02$; $\eta_p^2=0.077$).

After controlling for covariates, these differences remained significant for total vBMD and SSI and became significant for endosteal circumference and polar moment of inertia (IPOLAR) (Table 15). Total vBMD was higher ($p=0.001$; $\eta_p^2=0.135$) in Caucasians than East- ($p=0.006$) and South-Asian women ($p=0.001$), and SSI was higher ($p=0.04$; $\eta_p^2=0.058$) in Caucasians compared to South-Asian women ($p=0.04$). Endosteal circumference was higher in East-Asian than in Caucasian women (EA: 32.910 ± 0.881 vs. Cau: 32.544 ± 0.498 mm; $p=0.01$; $\eta_p^2=0.088$), whereas, IPOLAR was higher in East-Asians compared to South-Asian women (EA: 19057.207 ± 1037.106 vs. SA: 18195.017 ± 868.104 mm⁴; $p=0.04$; $\eta_p^2=0.064$).

Table 16 shows the results of pQCT measured bone variables, muscle CSA and density at 66% of non-dominant tibia sites, after controlling for height, weight, and duration of stay in the U.S. There were main effects of ethnicity for total BMC, cortical vBMD, BMC and area, IPLOAR and SSI, and of age for muscle density before controlling for covariates. Total BMC was higher in Caucasian than in South-Asian women (Cau: 363.015 ± 7.122 vs. SA: 320.435 ± 6.756 mg/mm; $p=0.001$; $\eta_p^2=0.127$). Cortical BMC (Cau: 328.802 ± 6.470 vs. SA: $288.950 \pm$

5.843 mg/mm; $p=0.001$; $\eta_p^2=0.127$) and area (Cau: 284.038 ± 5.690 vs. SA: 250.691 ± 5.448 mm²; $p=0.002$; $\eta_p^2=0.117$) were greater in Caucasians compared to South-Asians, while vBMD (EA: 1166.629 ± 2.757 vs. SA: 1153.739 ± 3.618 mg/cm³; $p=0.03$; $\eta_p^2=0.069$) was greater in East- than in South-Asian women. Both IPOLAR (Cau: 39789.565 ± 1545.599 vs. SA: 32682.946 ± 1444.722 mm⁴; $p=0.01$; $\eta_p^2=0.082$) and SSI (Cau: 2280.663 ± 68.054 vs. SA: 1923.628 ± 60.719 mm³; $p=0.006$; $\eta_p^2=0.096$) were significantly higher in Caucasian than in South-Asian women. Based on age groups, younger women had higher muscle density in comparison to middle-aged women (Young: 78.93 ± 0.19 vs. Middle-Aged: 77.79 ± 0.24 mg/cm³; $p=0.001$; $\eta_p^2=0.118$).

Following adjustment for covariates, the results retained significance for total and cortical BMC, cortical area, and muscle density, and gained significance for Ethnicity X Age interactions for periosteal and endosteal circumference (Table 16). For the main effects of ethnicity, total BMC was now higher in East-Asian compared to South-Asian women (EA: 331.554 ± 8.836 vs. SA: 320.435 ± 6.756 mg/mm; $p=0.02$), whereas, cortical BMC was higher in both Caucasian and East-Asian women compared to South-Asians (Cau: 328.802 ± 6.470 vs. SA: 288.950 ± 5.843 ; $p=0.03$; EA: 304.525 ± 8.205 vs. SA: 288.950 ± 5.843 mg/mm; $p=0.007$). The cortical area was significantly greater in Caucasians in contrast to both East- (Cau: 284.038 ± 5.690 vs. EA: 261.172 ± 7.16 mm²; $p=0.01$) and South-Asians (Cau: 284.038 ± 5.690 vs. SA: 250.691 ± 5.448 mm²; $p=0.04$). Based on age, muscle density was still greater in younger than in middle-aged women ($p=0.04$; $\eta_p^2=0.050$). Periosteal circumference was significantly greater ($p=0.04$; $\eta_p^2=0.065$) in middle-aged East-Asians compared to middle-aged Caucasians ($p=0.02$) and South-Asians ($p=0.02$), and endosteal circumference was greater

($p=0.04$; $\eta_p^2=0.066$) in middle-aged East-Asians compared to middle-aged Caucasian women
($p=0.01$).

Table 14. Bone characteristics at 4% of the non-dominant tibia for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean ± SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Total vBMC (mg/mm)	294.146 ± 9.679	314.777 ± 10.826	284.576 ± 7.418	275.120 ± 20.654	276.066 ± 8.764	288.779 ± 10.894
Total vBMD (mg/cm ³)	306.492 ± 6.169	313.368 ± 7.505	324.908 ± 9.632	282.710 ± 14.751	308.565 ± 11.162	328.436 ± 15.624
Trab BMC (mg/mm)	198.195 ± 7.100	211.256 ± 8.573	202.692 ± 6.578	195.086 ± 20.043	190.594 ± 9.259	210.734 ± 9.873
Trab vBMD (mg/cm ³) ^δ	253.950 ± 5.185	256.391 ± 7.154	270.617 ± 6.751	233.160 ± 15.938	249.471 ± 10.559	272.282 ± 11.327
Total Area (mm ²)	959.92 ± 25.06	1004.46 ± 25.95	887.84 ± 28.06	965.76 ± 27.30	907.36 ± 34.68	897.90 ± 50.32
Trab Area (mm ²)	779.71 ± 21.80	822.55 ± 23.50	757.99 ± 30.69	823.92 ± 28.60	772.07 ± 33.80	790.99 ± 52.68
Peri C (mm)	109.618 ± 1.424	112.152 ± 1.455	105.335 ± 1.635	110.066 ± 1.549	106.485 ± 1.987	105.770 ± 3.101
BSI (mg*mm)	90.976 ± 4.427	99.804 ± 5.229	93.139 ± 4.444	80.371 ± 10.566	85.919 ± 5.115	94.826 ± 5.994
Trab BSI (mg*mm)	50.856 ± 2.583	55.103 ± 3.433	55.015 ± 2.401	48.264 ± 8.909	48.530 ± 4.127	57.041 ± 3.084

^δ p<0.05, ^{δδ} p<0.01 Significant Ethnicity X Age interaction. vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; BSI, bone-strength index.

Table 15. Bone characteristics at 38% of the non-dominant tibia for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean \pm SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Total vBMC (mg/mm)	333.10 \pm 10.14	342.38 \pm 9.73	297.38 \pm 8.67	320.49 \pm 21.55	292.17 \pm 7.96	304.57 \pm 11.60
Total vBMD (mg/cm ³) ^{††}	948.65 \pm 8.93 ^{ββ, γγ}	954.91 \pm 8.13 ^{ββ, γγ}	927.75 \pm 13.91	898.70 \pm 19.94	899.28 \pm 15.32	919.83 \pm 15.91
Peri C (mm)	66.28 \pm 0.95	67.01 \pm 0.97	63.41 \pm 0.96	66.63 \pm 1.94	63.90 \pm 0.97	64.50 \pm 1.51
Endo C (mm) ^{††}	32.45 \pm 0.70	32.65 \pm 0.72	31.80 \pm 1.05 ^α	35.56 \pm 1.34 ^α	33.63 \pm 1.14	32.78 \pm 1.61
∞ Cort vBMC (mg/mm)	318.66 \pm 9.73	328.35 \pm 9.49	286.71 \pm 8.55	305.78 \pm 21.75	279.55 \pm 7.54	293.32 \pm 10.88
Cort vBMD (mg/cm ³)	1196.067 \pm 3.546	1202.645 \pm 4.187	1199.225 \pm 3.249	1204.550 \pm 5.035	1193.976 \pm 5.299	1196.736 \pm 5.706
Cort Area (mm ²)	266.54 \pm 8.28	273.15 \pm 7.96	239.17 \pm 7.28	254.03 \pm 18.26	234.52 \pm 7.11	245.31 \pm 9.59
SSI (mm ³) [†]	1475.43 \pm 61.67 ^γ	1537.85 \pm 63.09 ^γ	1290.63 \pm 51.95	1498.62 \pm 126.55	1285.02 \pm 51.89	1330.54 \pm 84.87
IPOLAR (mm ⁴) [†]	21241.12 \pm 1157.2	21894.59 \pm 1242.8	17881.95 \pm 1026.7 ^γ	21877.82 \pm 2385.5 ^γ	17634.61 \pm 965.1	19061.11 \pm 1658.9

[†]p<0.05, ^{††}p<0.01, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian; p<0.01, ^{αα} Compared to Caucasians, ^{ββ} Compared to East-Asian, ^{γγ} Compared to South-Asian). vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; Endo, endosteal; Cort, cortical; SSI, stress-strain index; IPLOAR, polar moment of inertia.

Table 16. Bone characteristics at 66% of the non-dominant tibia for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean ± SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Total vBMC (mg/mm) [†]	356.97 ± 9.07	369.61 ± 11.18	323.61 ± 8.77 ^γ	350.62 ± 21.07 ^γ	317.15 ± 8.72	325.51 ± 11.01
Total vBMD (mg/cm ³)	698.70 ± 10.69	729.445 ± 20.55	717.26 ± 17.21	659.180 ± 20.66	669.194 ± 16.49	699.536 ± 26.65
Peri C (mm) ^δ	80.08 ± 0.99	80.10 ± 1.71	75.51 ± 1.38	81.61 ± 2.38	77.25 ± 1.07	76.81 ± 2.12
Endo C (mm) ^δ	53.728 ± 0.99	52.658 ± 2.07	49.64 ± 1.61	56.98 ± 2.16	53.34 ± 1.38	51.57 ± 2.71
Cort vBMC (mg/mm) ^{††}	323.82 ± 8.73 ^γ	334.24 ± 9.69 ^γ	298.63 ± 8.51 ^{γγ}	318.66 ± 19.07 ^{γγ}	285.46 ± 7.75	294.34 ± 9.02
Cort vBMD (mg/cm ³)	1154.158 ± 3.808	1162.823 ± 5.606	1164.763 ± 3.403	1171.110 ± 4.538	1154.635 ± 4.646	1152.355 ± 6.023
Cort Area (mm ²) ^{††}	280.68 ± 7.70 ^γ	287.70 ± 8.55 ^γ	256.49 ± 7.45 ^γ	272.40 ± 16.71 ^γ	247.51 ± 7.28	255.62 ± 8.29
SSI (mm ³)	2266.38 ± 97.47	2296.24 ± 96.84	1926.66 ± 84.01	2299.28 ± 198.70	1892.92 ± 59.13	1971.09 ± 127.76
IPOLAR (mm ⁴)	39221.7 ± 2138.8	40409.0 ± 2280.9	31305.4 ± 1846.5	39542.6 ± 4501.2	32469.3 ± 1554.3	33013.2 ± 2890.1
MCSA (mm ²)	6135.96 ± 269.69	6248.92 ± 333.32	6617.20 ± 302.75	6712.67 ± 221.78	6385.89 ± 317.95	6528.45 ± 410.63
Muscle Density (mg/cm ³) [*]	78.92 ± 0.21	77.49 ± 0.41	79.12 ± 0.34	78.380 ± 0.438	78.76 ± 0.31	77.51 ± 0.49

*p<0.05, **p<0.01, Significant age difference (Young vs. Middle-aged); [†]p<0.05, ^{††}p<0.01, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian; p<0.01, ^{αα} Compared to Caucasians, ^{ββ} Compared to East-Asian, ^{γγ} Compared to South-Asian). vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; Endo, endosteal; Cort, cortical; SSI, stress-strain index; IPLOAR, polar moment of inertia; MCSA, muscle cross-sectional area.

Muscle Function Assessment

Table 17 represents the results for muscle performance variables after adjustment for covariates of height, weight and duration of stay in the U.S. No significant main effects of age or Ethnicity X Age interactions were noted before or after adjustment for covariates.

Before controlling for covariates, handgrip strength for both the right (Cau: 26.39 ± 0.65 vs. SA: 22.81 ± 0.83 kg; $p=0.003$; $\eta_p^2=0.109$) and left (Cau: 24.05 ± 0.67 vs. SA: 21.24 ± 0.87 kg; $p=0.03$; $\eta_p^2=0.067$) sides was significantly greater in Caucasian compared to South-Asian women. Jump height (Cau: 12.35 ± 0.45 , EA: 12.15 ± 0.44 vs. SA: 9.94 ± 0.42 inches; $p=0.001$; $\eta_p^2=0.145$) and time in air (Cau: 0.50 ± 0.01 , EA: 0.50 ± 0.01 vs. SA: 0.45 ± 0.01 s; $p=0.001$; $\eta_p^2=0.155$) were significantly greater for both Caucasian and East-Asian women in comparison to South-Asians, whereas, jump power was higher in Caucasians and South-Asians compared to East-Asian women (Cau: 859.61 ± 38.62 , SA: 881.37 ± 43.70 vs. EA: 712.08 ± 27.49 watts; $p=0.006$; $\eta_p^2=0.096$). 1-RM leg press strength was significantly higher in Caucasians in contrast to East- and South-Asian women (Cau: 144.96 ± 6.27 vs. EA: 120.03 ± 6.58 , SA: 119.71 ± 4.82 kg; $p=0.002$; $\eta_p^2=0.115$).

Following adjustment for covariates, the main effects of ethnicity were retained 1 RM leg press, jump height, and time in air (Table 17). 1-RM leg press was significantly higher ($p=0.04$; $\eta_p^2=0.061$) for Caucasian compared to South-Asian women ($p=0.04$). Additionally, main effects of age were noted for jump height (Young: 12.16 ± 0.36 vs. Middle-aged: 10.81 ± 0.44 inches; $p=0.03$; $\eta_p^2=0.049$) and time (Young: 0.49 ± 0.01 vs. Middle-aged: 0.47 ± 0.01 s; $p=0.04$; $\eta_p^2=0.041$), both of which were higher in younger in comparison to middle-aged women.

Table 17. Muscle performance variables for young and middle-aged Caucasian, East-Asian, and South-Asian women (Mean \pm SE)

Variables	Caucasian (n=46)		East-Asian (n=34)		South-Asian (n=27)	
	Young (n= 24)	Middle-Aged (n= 22)	Young (n= 24)	Middle-Aged (n= 10)	Young (n= 17)	Middle-Aged (n= 10)
Right Handgrip (kg)	26.01 \pm 0.88	26.80 \pm 0.97	22.87 \pm 0.88	25.73 \pm 1.43	23.25 \pm 1.15	22.14 \pm 1.19
Left Handgrip (kg)	24.09 \pm 0.89	24.02 \pm 1.03	20.85 \pm 0.89	24.42 \pm 1.48	21.80 \pm 1.12	20.36 \pm 1.39
Time in air (s) *	0.51 \pm 0.01	0.48 \pm 0.01	0.50 \pm 0.01	0.50 \pm 0.02	0.46 \pm 0.01	0.42 \pm 0.01
Jump Height (inches) *	13.16 \pm 0.67	11.46 \pm 0.55	12.10 \pm 0.54	12.24 \pm 0.80	10.59 \pm 0.55	8.95 \pm 0.58
Velocity (m/s)	1.24 \pm 0.03	1.27 \pm 0.03	1.27 \pm 0.02	1.24 \pm 0.04	1.28 \pm 0.03	1.23 \pm 0.04
Jump Power (watts)	786.69 \pm 49.1	939.17 \pm 56.9	712.11 \pm 35.3	712.00 \pm 41.9	847.63 \pm 53.9	933.52 \pm 74.0
Relative Jump Power (watts/kg)	12.08 \pm 0.41	12.36 \pm 0.33	12.43 \pm 0.24	12.17 \pm 0.44	12.59 \pm 0.27	12.23 \pm 0.46
1 RM (kg) [†]	147.05 \pm 9.37 ^γ	143.60 \pm 8.43 ^γ	124.82 \pm 7.70	108.55 \pm 12.46	124.60 \pm 7.04	113.36 \pm 6.22

*p<0.05, **p<0.01, Significant age difference (Young vs. Middle-aged); [†]p<0.05, ^{††}p<0.01, Significant ethnicity difference (p<0.05, ^αCompared to Caucasians, ^β Compared to East-Asian, ^γ Compared to South-Asian; p<0.01, ^{αα} Compared to Caucasians, ^{ββ} Compared to East-Asian, ^{γγ} Compared to South-Asian). 1 RM, 1 Repetition Maximum.

Correlations

Zero-order Pearson Product Moment correlations were computed to determine relationships between dependent variables separately for the three racial/ethnic groups, Caucasians, East-Asians, and South-Asians. Relationships were determined between participant characteristics such as age, height, weight, age of menarche and duration of stay in U.S., physical activity scores, calcium and vitamin D intakes, body composition, muscle performance variables, muscle cross-sectional area and density, and areal BMD measurements at total body, lumbar spine, femoral neck, trochanter, and total hip, and volumetric BMD and strength measurements performed at 4, 38, and 66% of non-dominant tibia. Although correlation coefficients were computed for all dependent variables, the tables outline only the coefficients with statistically significant ($p < 0.05$) relationships (Table 18-29).

Tables 18-20 summarize the correlation coefficients for DXA measured areal BMD values for Caucasians, East-Asians, and South-Asians respectively. There was a moderately positive relationship between age, anthropometric variables and DXA measured areal BMD for Caucasians (Table 18). For East-Asians, these relationships were most prevalent between areal BMD and lean mass and muscle strength (Table 19), while for South-Asians areal BMD was positively related to body composition and jump test variables (Table 20).

Tables 21-23 represent the correlation coefficient for Caucasians, East-Asians, and South-Asians at 4% of the non-dominant tibia, whereas tables 24-26, and tables 27-29 represent the correlation coefficients at 38% and 66% of the non-dominant tibia for Caucasians, East-Asians, and South-Asians respectively. At 4% of the tibia site, pQCT measured bone density and strength variables were positively related to physical activity, age of menarche, body composition, and muscle strength for Caucasians (Table 21) and South-Asians (Table 23). For

East-Asians, this relationship was evident with bone free lean mass, muscle strength and physical activity (Table 22). Height, weight, muscle strength, body composition, and age of menarche were significantly related to pQCT measured bone density and strength variables at 38% for all the three ethnicities (Table 24-26). At 66% of tibia sites, body composition and physical activity were positively related to vBMD and strength variables assessed using pQCT for Caucasians (Table 27) and East-Asians (Table 28). For South-Asians this relationship could be seen between vBMD and bone strength variables and age of menarche, physical activity, body composition and muscle strength (Table 29).

Table 18. Correlations between areal BMD at total body, lumbar spine, trochanter, total hip and physical characteristics for Caucasians

	Total body aBMD (g/cm ²)	L1-L4 aBMD (g/cm ²)	FN aBMD (g/cm ²)	Troch aBMD (g/cm ²)	THIP aBMD (g/cm ²)
Age (years)	0.45**	0.08	-0.17	-0.08	-0.05
Height (cm)	0.52**	0.36*	0.26	0.29	0.26
Weight (kg)	0.69**	0.42**	0.41**	0.52**	0.60**
Menarche Age (years)	-0.33*	-0.54**	-0.01	-0.32*	-0.23
U.S. Residency (years)	0.42**	0.15	-0.10	0.02	0.001
Handgrip (kg)	0.39**	0.39**	0.26	0.35*	0.31*
Jump Power (watts)	0.64**	0.45**	0.35*	0.50**	0.57**
1 RM (kg)	0.35*	0.37*	0.26	0.39**	0.35*
Body Fat %	0.36*	0.13	0.22	0.21	0.35*
A/G Ratio	0.50**	0.26	0.29*	0.41**	0.51**
eVAT mass (g)	0.39**	0.16	0.15	0.26	0.38**
Fat mass (kg)	0.56**	0.29	0.32*	0.39**	0.51**
BFLBM (kg)	0.80**	0.61**	0.52**	0.61**	0.63**

*p<0.05, **p<0.01. aBMD, areal bone mineral density; L1-L4, lumbar vertebrae 1 to 4; FN, femur neck; Troch, trochanter, THIP, total hip; U.S., United States; A/G, Android/Gynoid; eVAT, estimated visceral adipose tissue; BFLBM, bone free lean body mass; 1-RM, 1 Repetition Maximum;

Table 19. Correlations between areal BMD at total body, lumbar spine, trochanter, total hip and physical characteristics for East-Asians

	Total body aBMD (g/cm ²)	L1-L4 aBMD (g/cm ²)	FN aBMD (g/cm ²)	Troch aBMD (g/cm ²)	THIP aBMD (g/cm ²)
Age (years)	0.18	0.17	-0.40*	-0.37*	-0.35*
Vitamin D Intake (IU/day)	-0.17	-0.22	-0.34*	-0.25	-0.26
Handgrip (kg)	0.30	0.38*	0.06	0.11	0.09
Jump Power (watts)	0.39*	0.26	0.23	0.23	0.18
1 RM (kg)	0.47**	0.31	0.27	0.42*	0.38*
BFLBM (kg)	0.49**	0.42*	0.26	0.33	0.24

*p<0.05, **p<0.01. aBMD, areal bone mineral density; L1-L4, lumbar vertebrae 1 to 4; FN, femur neck; Troch, trochanter, THIP, total hip; BFLBM, bone free lean body mass; 1 RM, 1 Repetition Maximum;

Table 20. Correlations between areal BMD at total body, lumbar spine, trochanter, total hip and physical characteristics for South-Asians

	Total body aBMD (g/cm ²)	L1-L4 aBMD (g/cm ²)	FN aBMD (g/cm ²)	Troch aBMD (g/cm ²)	THIP aBMD (g/cm ²)
Age (years)	0.31	0.41*	0.30	0.27	0.23
Weight (kg)	0.13	0.36	0.61**	0.61**	0.59**
tBPAQ Score	0.29	0.52**	0.34	0.18	0.27
U.S. Residency (years)	0.25	0.42*	-0.07	-0.03	-0.05
Jump Power (watts)	0.09	0.26	0.49**	0.50**	0.47*
Jump Height (inches)	-0.07	-0.11	-0.31	-0.39*	-0.34
Time in air (s)	-0.10	-0.17	-0.38*	-0.43*	-0.40*
Body Fat %	0.04	0.31	0.62**	0.62**	0.67**
A/G Ratio	0.25	0.32	0.54**	0.51**	0.47*
eVAT mass (g)	0.14	0.46*	0.67**	0.63**	0.62**
Fat mass (kg)	0.09	0.34	0.65**	0.64**	0.65**
BFLBM (kg)	0.16	0.32	0.45*	0.48*	0.42*
Muscle Density (mg/mm ³)	-0.40*	-0.36	-0.39*	-0.46*	-0.42*

*p<0.05, **p<0.01. aBMD, areal bone mineral density; L1-L4, lumbar vertebrae 1 to 4; FN, femur neck; Troch, trochanter, THIP, total hip; tBPAQ, total bone specific physical activity questionnaire; A/G, Android/Gynoid; eVAT, estimated visceral adipose tissue; BFLBM, bone free lean body mass; 1- RM, 1 Repetition Maximum;

Table 21. Correlations between 4% pQCT variables and physical characteristics for Caucasians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Trab BMC (mg/mm)	Trab vBMD (mg/cm ³)	Total Area (mm ²)	Trab Area (mm ²)	Peri C (mm)	BSI (mg*mm)	Trab BSI (mg*mm)
Height (cm)	0.59**	0.33*	0.44**	0.24	0.53**	0.43**	0.53**	0.53**	0.37*
Weight (kg)	0.62**	0.47**	0.46**	0.35*	0.44**	0.35*	0.44**	0.61**	0.44**
Handgrip (kg)	0.50**	0.11	0.43**	0.14	0.57**	0.48**	0.57**	0.34*	0.33*
Jump Power (watts)	0.61**	0.36*	0.43**	0.26	0.50**	0.38**	0.50**	0.55**	0.39**
1 RM (kg)	0.49**	0.22	0.57**	0.39**	0.47**	0.46**	0.47**	0.41**	0.53**
Body Fat %	0.28	0.29	0.15	0.17	0.13	0.08	0.13	0.31*	0.16
A/G Ratio	0.40**	0.32*	0.34*	0.27	0.26	0.24	0.26	0.40**	0.33*
Fat mass (kg)	0.49**	0.43**	0.31*	0.28	0.29*	0.21	0.29*	0.50**	0.31*
BFLBM (kg)	0.67**	0.42**	0.57**	0.38**	0.53**	0.47**	0.55**	0.62**	0.53**
eVAT mass (g)	0.29	0.29	0.18	0.16	0.14	0.12	0.15	0.31*	0.18
Menarche (years)	-0.35*	-0.16	-0.39*	-0.19	-0.31*	-0.27	-0.31*	-0.31*	-0.31*
Total PA score (mets/min)	0.28	0.002	0.27	0.05	0.38**	0.35*	0.38**	0.17	0.19
MCSA (mm ²)	0.03	-0.27	0.12	-0.18	0.28	0.31*	0.27	-0.09	0.01

*p<0.05, **p<0.01. vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; BSI, bone-strength index; A/G, Android/Gynoid; eVAT, estimated visceral adipose tissue; BFLBM, bone free lean body mass; 1- RM, 1 Repetition Maximum; PA, physical activity; MCSA, muscle cross-sectional area.

Table 22. Correlations between 4% pQCT variables and physical characteristics for East-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Trab BMC (mg/mm)	Trab vBMD (mg/cm ³)	Peri C (mm)	BSI (mg*mm)	Trab BSI (mg*mm)
Weight (kg)	0.47**	0.26	0.36*	0.28	0.23	0.40*	0.37*
Handgrip (kg)	0.26	-0.09	0.15	-0.12	0.34*	0.13	0.10
Jump Power (watts)	0.45**	0.28	0.29	0.28	0.17	0.40*	0.33
1 RM (kg)	0.44**	0.29	0.39*	0.35*	0.12	0.43*	0.42*
Age (years)	-0.08	-0.40*	-0.02	-0.41*	0.32	-0.23	-0.14
BFLBM (kg)	0.66**	0.25	0.54**	0.32	0.44**	0.51**	0.51**
tBPAQ score	0.29	0.29	0.30	0.38*	0.00	0.32	0.37*

*p<0.05, **p<0.01. vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; BSI, bone-strength index; BFLBM, bone free lean body mass; tBPAQ, total bone specific physical activity questionnaire.

Table 23. Correlations between 4% pQCT variables and physical characteristics for South-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Trab BMC (mg/mm)	Trab vBMD (mg/cm ³)	Total Area (mm ²)	Trab Area (mm ²)	Peri C (mm)	BSI (mg*mm)	Trab BSI (mg*mm)
Height (cm)	0.11	-0.40*	0.15	-0.42*	0.53**	0.56**	0.50**	-0.22	-0.17
Weight (kg)	0.58**	0.21	0.46*	0.22	0.24	0.24	0.25	0.46*	0.42*
Jump Power (watts)	0.54**	0.16	0.41*	0.18	0.26	0.24	0.26	0.40*	0.36
1 RM (kg)	0.45*	0.17	0.27	0.16	0.23	0.17	0.21	0.34	0.24
Age (years)	0.20	0.10	0.37	0.22	0.06	0.18	0.06	0.16	0.32
Body Fat %	0.35	0.54**	0.27	0.55**	-0.27	-0.24	-0.25	0.55**	0.48**
A/G Ratio	0.45*	0.42*	0.50**	0.51**	-0.06	0.03	-0.06	0.50**	0.57**
Fat mass (kg)	0.50**	0.36	0.39*	0.37	0.02	0.03	0.03	.52**	0.46*
BFLBM (kg)	0.62**	-0.04	0.50**	-0.01	0.52**	0.49**	0.52**	0.32	0.31
eVAT mass (g)	0.43*	0.37	0.42*	0.41*	-0.04	0.03	-0.03	0.47*	0.48*
Menarche (years)	0.18	-0.40*	0.25	-0.37	0.56**	0.60**	0.54**	-0.19	-0.08
Total PA score (mets/min)	0.53**	0.20	0.28	0.18	0.21	0.12	0.22	0.45*	0.27

*p<0.05, **p<0.01. vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density; Trab, trabecular; Peri, periosteal; BSI, bone-strength index; A/G, Android/Gynoid; eVAT, estimated visceral adipose tissue; BFLBM, bone free lean body mass; PA, physical activity.

Table 24. Correlations between 38% pQCT variables and physical characteristics for Caucasians

	Total BMC (mg/mm)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort Area (mm ²)	Peri C (mm)	Endo C (mm)	IPolar (mm ⁴)	SSI (mm ³)
Height (cm)	0.57**	0.61**	0.53**	0.53**	0.60**	0.49**	0.59**	0.61**
Weight (kg)	0.50**	0.47**	0.49**	0.51**	0.47**	0.18	0.46**	0.47**
Handgrip (kg)	0.55**	0.52**	0.56**	0.54**	0.51**	0.24	0.57**	0.53**
Jump Power (watts)	0.53**	0.50**	0.53**	0.53**	0.51**	0.23	0.51**	0.52**
1 RM (kg)	0.41**	0.41**	0.40**	0.42**	0.42**	0.20	0.39**	0.41**
Age (years)	0.07	0.06	0.09	0.07	0.06	0.03	0.06	0.09
A/G Ratio	0.25	0.24	0.26	0.30*	0.25	0.02	0.22	0.23
Fat mass (kg)	0.32*	0.30*	0.31*	0.33*	0.31*	0.11	0.28	0.31*
BFLBM (kg)	0.63**	0.57**	0.63**	0.63**	0.57**	0.18	0.59**	0.58**
Menarche (years)	-0.36*	-0.31*	-0.36*	-0.40*	-0.30*	-0.09	-0.34*	-0.32*
Serum vitamin D (ng/mL)	0.20*	0.81	0.18	0.17	0.08	-0.13	0.09	0.12
Total PA score (mets/min)	0.41**	0.34*	0.40**	0.40**	0.33*	0.04	0.38**	0.34*

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; SSI, bone-stress-strain index; A/G, Android/Gynoid; BFLBM, bone free lean body mass; 1 RM, 1 Repetition Maximum; PA, physical activity.

Table 25. Correlations between 38% pQCT variables and physical characteristics for East-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort vBMD (mg/cm ³)	Cort Area (mm ²)	Peri C (mm)	Endo C (mm)	IPolar (mm ⁴)	SSI (mm ³)
Height (cm)	0.27	-0.34	0.43*	0.22	-0.13	0.23	0.44**	0.51**	0.39*	0.38*
Weight (kg)	0.45**	-0.08	0.49**	0.42*	-0.43*	0.45**	0.49**	0.28	0.51**	0.46**
Handgrip (kg)	0.44*	0.05	0.40*	0.39*	-0.09	0.39*	0.39*	0.17	0.40*	0.40*
Jump Power (watts)	0.47**	0.06	0.45**	0.44**	-0.31	0.46**	0.44**	0.18	0.50**	0.44**
Jump Height (inch)	0.35*	0.48**	0.11	0.35*	0.01	0.35*	0.11	-0.32	0.18	0.15
Jump time (s)	0.34	0.48**	0.10	0.34	0.01	0.34	0.11	-0.32	0.17	0.14
1 RM (kg)	0.49**	0.49**	0.27	0.51**	-0.16	0.52**	0.26	-0.31	0.34	0.29
Fat mass (kg)	0.16	-0.17	0.24	0.14	-0.37*	0.16	0.24	0.23	0.25	0.22
BFLBM (kg)	0.70**	0.04	0.68**	0.67**	-0.41*	0.69**	0.69**	0.27	0.70**	0.66**
Vitamin D Intake (IU/day)	-0.34*	-0.12	-0.29	-0.33	0.32	-0.34*	-0.30	-0.03	-0.28	-0.26

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; SSI, bone-stress-strain index; BFLBM, bone free lean body mass; 1 RM, 1 Repetition Maximum.

Table 26. Correlations between 38% pQCT variables and physical characteristics for South-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort vBMD (mg/cm ³)	Cort Area (mm ²)	Peri C (mm)	Endo C (mm)	IPolar (mm ⁴)	SSI (mm ³)
Height (cm)	0.23	-.59**	.52**	0.20	-0.03	0.18	.516**	.631**	.477*	.487**
Weight (kg)	0.69**	0.03	0.58**	0.69**	-0.32	0.67**	0.58**	0.21	0.65**	0.56**
Handgrip (kg)	0.15	-0.39*	0.35	0.08	-0.17	0.10	0.33	0.43*	0.31	0.31
Jump Power (watts)	0.59**	0.06	0.48**	0.59**	-0.40*	0.59**	0.48**	0.13	0.55**	0.45*
1 RM (kg)	0.46*	-0.13	0.49**	0.43*	-0.37	0.44*	0.48**	0.29	0.53**	0.44*
Body Fat %	0.46*	0.45*	0.15	0.48**	-0.23	0.47*	0.16	-0.25	0.23	0.15
A/G Ratio	0.58**	0.19	0.41*	0.58**	-0.39*	0.59**	0.41*	0.02	0.48*	0.41*
Fat mass (kg)	0.65**	0.22	0.44*	0.66**	-0.30	0.64**	0.44*	0.02	0.52**	0.43*
BFLBM (kg)	0.66**	-0.22	0.69**	0.64**	-0.31	0.63**	0.69**	0.44*	0.74**	0.66**
eVAT mass (g)	0.62**	0.15	0.45*	0.63**	-0.22	0.60**	0.46*	0.09	0.53**	0.45*
Oral contraceptive use (years)	-0.39*	0.04	-0.36	-0.40*	0.40*	-0.41*	-0.38*	-0.13	-0.32	-0.33
Menarche (years)	0.10	-0.53**	0.39*	0.05	-0.06	0.05	0.38*	0.54**	0.35	0.35
Total PA (mets/min)	0.33	-0.17	0.37*	0.31	-0.18	0.30	0.38*	0.27	0.37	0.33
MCSA (mm ²)	-0.35	0.31	-0.45*	-0.34	0.19	-0.34	-0.46*	-0.37	-0.42*	-0.46*

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; SSI, bone-stress-strain index; 1 RM, 1 Repetition Maximum; A/G, Android/Gynoid; BFLBM, bone free lean body mass; eVAT, estimated visceral adipose tissue; PA, physical activity; MCSA, muscle cross-sectional area.

Table 27. Correlations between 66% pQCT variables and physical characteristics for Caucasians

	Total BMC (mg/mm)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort Area (mm ²)	Peri C (mm)
Height (cm)	0.61**	0.46**	0.61**	0.60**	0.45**
Weight (kg)	0.50**	0.33*	0.48**	0.51**	0.33*
Handgrip (kg)	0.55**	0.44**	0.55**	0.52**	0.43**
Jump Power (watts)	0.51**	0.45**	0.47**	0.48**	0.44**
Velocity (m/s)	0.21	0.36*	0.16	0.16	0.36*
1 RM (kg)	0.34*	0.28	0.34*	0.35*	0.28
A/G Ratio	0.28	0.11	0.26	0.29*	0.11
Fat mass (kg)	0.32*	0.18	0.31*	0.34*	0.18
BFLBM (kg)	0.62**	0.44**	0.59**	0.60**	0.44**
Menarche (years)	-0.31*	-0.23	-0.28	-0.25	-0.22
Total PA score (mets/min)	0.35*	0.26	0.37*	0.37*	0.26

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; 1 RM, 1 Repetition Maximum; A/G, Android/Gynoid; PA, physical activity.

Table 28. Correlations between 66% pQCT variables and physical characteristics for East-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort vBMD (mg/cm ³)	Cort Area (mm ²)	Peri C (mm)	Endo C (mm)	IPolar (mm ⁴)	SSI (mm ³)
Height (cm)	0.31	-0.30	0.43*	0.25	-0.21	0.27	0.44**	0.43*	0.19	0.18
Weight (kg)	0.46**	-0.09	0.42*	0.42*	-0.67**	0.47**	0.421*	0.29	0.14	0.16
Handgrip (kg)	0.40*	-0.17	0.38*	0.38*	-0.12	0.38*	0.39*	0.31	0.39*	0.41*
Jump Power (watts)	0.47**	0.04	0.34	0.43*	-0.56**	0.47**	0.34	0.18	0.10	0.10
1 RM (kg)	0.38*	0.28	0.12	0.42*	-0.28	0.44**	0.11	-0.11	0.14	0.13
Age (years)	0.26	-0.38*	0.42*	0.21	0.24	0.18	0.42*	0.47**	0.39*	0.39*
Fat mass (kg)	0.19	-0.14	0.23	0.13	-0.59**	0.18	0.23	0.21	-0.04	-0.03
BFLBM (kg)	0.70**	-0.01	0.55**	0.68**	-0.62**	0.72**	0.56**	0.32	0.33	0.35*
Vitamin D Intake (IU/day)	-0.28	-0.01	-0.20	-0.26	0.55**	-0.30	-0.21	-0.10	-0.21	-0.24

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; SSI, bone-stress-strain index; 1 RM, 1 Repetition Maximum; BFLBM, bone free lean body mass.

Table 29. Correlations between 66% pQCT variables and physical characteristics for South-Asians

	Total BMC (mg/mm)	Total vBMD (mg/cm ³)	Total Area (mm ²)	Cort BMC (mg/mm)	Cort vBMD (mg/cm ³)	Cort Area (mm ²)	Peri C (mm)	Endo C (mm)	IPolar (mm ⁴)	SSI (mm ³)
Height (cm)	0.21	-0.43*	0.54**	0.10	-0.02	0.09	0.54**	0.54**	0.51**	0.48**
Weight (kg)	0.68**	0.11	0.47*	0.63**	-0.46*	0.65**	0.47*	0.20	0.60**	0.57**
Handgrip (kg)	0.16	-0.17	0.30	0.09	-0.01	0.09	0.29	0.28	0.28	0.24
Jump Power (watts)	0.58**	0.14	0.38*	0.56**	-0.48**	0.59**	0.37	0.12	0.49**	0.46*
1 RM (kg)	0.48*	0.01	0.40*	0.47*	-0.31	0.48*	0.40*	0.20	0.44*	0.41*
Body Fat %	0.45*	0.45*	0.00	0.49**	-0.36	0.50**	0.01	-0.25	0.15	0.11
A/G Ratio	0.49**	0.29	0.19	0.49**	-0.52**	0.53**	0.18	-0.07	0.25	0.29
Fat mass (kg)	0.63**	0.26	0.30	0.61**	-0.45*	0.63**	0.30	0.02	0.41*	0.41*
BFLBM (kg)	0.66**	-0.14	0.65**	0.56**	-0.44*	0.59**	0.65**	0.43*	0.74**	0.72**
eVAT mass (g)	0.58**	0.22	0.29	0.54**	-0.41*	0.56**	0.29	0.04	0.38*	0.40*
tBPAQ Score	0.37	0.16	0.16	0.48*	0.04	0.43*	0.17	-0.03	0.29	0.32
Menarche (years)	0.13	-0.33	0.44*	0.03	0.02	0.02	0.42*	0.44*	0.37	0.42*
Muscle Density (mg/mm ³)	-0.18	-0.42*	0.17	-0.26	0.03	-0.24	0.17	0.33	0.10	0.01

*p<0.05, **p<0.01. BMC, bone mineral content; vBMD, volumetric bone mineral density; Cort, cortical; Peri, periosteal; SSI, bone-stress-strain index; 1 RM, 1 Repetition Maximum; A/G, Android/Gynoid; BFLBM, bone free lean body mass; eVAT, estimated visceral adipose tissue; tBPAQ, total bone specific physical activity questionnaire.

Multiple Regression Analyses

Tables 30-32 represent the results for forward regression analyses that were used to determine predictors of the lumbar spine and femoral neck areal BMD, and bone-strength and stress-strain indices for Caucasians, East-Asians and South-Asians respectively. The independent variables entered in the regression model included fat mass, BFLBM, total BPAQ, and average handgrip strength, which were chosen based on prior literature and the correlations found in this study. Our sample size for each ethnicity (Cau=46; EA=34; SA=27) is not sufficient to support five explanatory variables and it leads to violation of the thumb rule which dictates that in multiple regression 20 data points are required for each predictor. These small sample sizes increase our chances of overfitting the regression model and decreases the statistical power. However, our purpose of using regression is not to test the hypotheses for this study or to obtain a prediction equation, but to examine whether predictors of areal BMD and bone strength parameters vary as per ethnicity. If they do, then we suggest future large-scale studies to confirm that hypothesis.

Our results indicated that BFLBM was a positive, and age of menarche was a negative predictor of lumbar spine (LS) BMD for Caucasians (adj. $R^2=0.464$) ($p=0.003$), while handgrip strength (adj. $R^2=0.163$) ($p=0.02$) and total BPAQ (adj. $R^2=0.224$) ($p=0.007$) predict LS BMD in East-Asians and South-Asians respectively. Bone free lean body mass (adj. $R^2=0.263$) ($p=0.001$) was a significant predictor of the femoral neck (FN) BMD in Caucasians, and fat mass (adj. $R^2=0.410$) ($p=0.001$) predicted FN BMD in South-Asians. For East-Asians, these predictors did not fit the multiple regression model for FN BMD. Bone free lean body mass (adj. $R^2=0.368$) ($p=0.001$) also predicted bone-strength index at 4% of the tibia for Caucasians and East-Asians, along with total BPAQ (adj. $R^2=0.233$) ($p=0.006$), whereas, for South-Asians, fat

mass was a significant predictor of BSI. In Caucasians, BFLBM (adj. $R^2=0.326$) ($p=0.001$) was a predictor for stress-strain index at 38% of the tibia, while no predictor could fit the model for 66% of the tibia. In East-Asians, stress-strain indices were predicted by BFLBM and total BPAQ at 38% (adj. $R^2=0.566$) ($p=0.003$) and handgrip strength at 66% (adj. $R^2=0.135$) ($p=0.03$) of tibia sites, while in South-Asians, they were predicted by BFLBM at both the sites (38% tibia: adj. $R^2=0.407$) ($p=0.001$); (66% tibia: adj. $R^2=0.499$) ($p=0.001$).

Table 30. Results for forward regression analysis for Caucasians

	Value	Coefficient \pm SE	Standardized Coefficient	P-value
LS BMD (g/cm ²)	Intercept	1.37 \pm 0.16		0.001
	BFLBM (kg)	0.007 \pm 0.002	0.46	0.001
	Age of Menarche (years)	-0.03 \pm 0.01	-0.37	0.003
FN BMD (g/cm ²)	Intercept	0.75 \pm 0.07		0.001
	BFLBM (kg)	0.007 \pm 0.002	0.53	0.001
BSI (4% tibia) (mg*mm)	Intercept	17.89 \pm 15.08		0.242
	BFLBM (kg)	1.83 \pm 0.35	0.62	0.001
SSI (38% tibia) (mm ³)	Intercept	182.63 \pm 230.25		0.432
	BFLBM (kg)	16.81 \pm 4.69	0.44	0.001
	Handgrip strength (kg)	24.29 \pm 8.56	0.35	0.007

BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; BSI, bone strength index; SSI, stress-strain index, BFLBM, bone free lean body mass

Table 31. Results for forward regression analysis for East-Asians

	Value	Coefficient \pm SE	Standardized Coefficient	P-value
LS BMD (g/cm ²)	Intercept	0.93 \pm 0.10		0.001
	Handgrip strength (kg)	0.011 \pm 0.004	0.44	0.02
BSI (4% tibia) (mg*mm)	Intercept	-21.14 \pm 28.53		0.465
	BFLBM (kg)	2.88 \pm 0.77	0.57	0.001
	tBPAQ	0.36 \pm 0.14	0.40	0.02
SSI (38% tibia) (mm ³)	Intercept	-308.72 \pm 287.37		0.293
	BFLBM (kg)	43.61 \pm 7.73	0.71	0.001
	tBPAQ	4.48 \pm 1.365	0.41	0.003
SSI (66% tibia) (mm ³)	Intercept	963.38 \pm 470.63		0.05
	Handgrip strength (kg)	47.05 \pm 20.28	0.41	0.03

BMD, bone mineral density; LS, lumbar spine; BSI, bone strength index; SSI, stress-strain index, BFLBM, bone free lean body mass; tBPAQ, total bone specific physical activity questionnaire.

Table 32. Results for forward regression analysis for South-Asians

	Value	Coefficient \pm SE	Standardized Coefficient	P-value
LS BMD (g/cm ²)	Intercept	1.16 \pm 0.03		0.001
	tBPAQ	0.005 \pm 0.002	0.50	0.007
FN BMD (g/cm ²)	Intercept	0.74 \pm 0.06		0.001
	Fat mass (kg)	0.009 \pm 0.002	0.66	0.001
BSI (4% tibia) (mg*mm)	Intercept	56.02 \pm 11.85		0.001
	Fat mass (kg)	1.15 \pm 0.38	0.51	0.006
SSI (38% tibia) (mm ³)	Intercept	399.82 \pm 213.37		0.07
	BFLBM (kg)	23.53 \pm 5.42	0.66	0.001
SSI (66% tibia) (mm ³)	Intercept	610.04 \pm 260.39		0.00
	BFLBM (kg)	34.27 \pm 6.61	0.72	0.001

BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; BSI, bone strength index; SSI, stress-strain index, BFLBM, bone free lean body mass; tBPAQ, total bone specific physical activity questionnaire.

DISCUSSION

The aim of this cross-sectional study was to examine differences in bone status, bone free lean body mass, fat mass, and muscle strength in young and middle-aged premenopausal women belonging to Caucasian, East-Asian and South-Asian descents. A total of 107 recreationally active women completed the entire study. Participants were categorized into one of the three ethnicities based on the ethnicity of three out of four of their biological grandparents. For each ethnicity, the given age range (18-45 years) was further subdivided into two groups: young (18-30 years); and middle-aged (>30-45 years). This allowed comparison between women who are accruing bone mass versus those who have already achieved their peak bone mass. Additionally, this study also evaluated differences in physical activity, calcium and vitamin D intakes, and serum vitamin D levels in these participants.

The Asian ethnicity is geographically and culturally diverse and is comprised of East-Asian, South-Asian, North-Asian, Southeast-Asian, West-Asian, and Central-Asian sub-groups. Previous studies have documented that the diagnosis of osteoporosis and fracture incidence varies among Asian subgroups (Cheung et al., 2018). Although East-Asians are documented to have a lower areal BMD; their non-axial fracture rates are lower than those of Caucasian women (Cong & Walker, 2014). Unlike East-Asians, South-Asian women are known to have a higher incidence of osteoporotic fractures and a 10-20 years earlier diagnosis of osteoporosis (Makker et al., 2008). However, most of the studies conducted in the United States examining bone mineral density and its predictors focus on East-Asian women or combine all Asians into one category and do not classify them by their sub-groups.

Studies assessing racial/ethnic differences in bone density and its predictors often consist of both native and immigrant participants, especially in the non-White categories. However,

these studies fail to control for variations that may arise due to cultural and geographical differences amongst participants belonging to different ethnicities or amongst native and immigrant participants belonging to the same ethnicity. Our current sample for this study consisted of both immigrant (39.3%) and the U.S. born (60.7%) participants, resulting in differences in duration the participants have stayed in U.S. (Table 5, Table 7). Talegawkar et al. (2016), emphasized the concept of “acculturation” and its role in modifying lifestyle behaviors such as diet and physical activity and their impact on health outcomes in immigrant populations. Merriam-Webster’s Collegiate Dictionary defines acculturation as “*cultural modification of an individual, group, or people by adapting to or borrowing traits from another culture; also, a merging of cultures as a result of prolonged contact.*” The process of acculturation ranges from exposure of the individual to a new and different culture, and gradual willingness of the individual to adapt traits from that culture and merge them with existing traits from the previous culture, to finally a change in attitudes, behaviors, beliefs and lifestyle practices including dietary preferences, activity behaviors, choice of music, etc. (Cuellar, Arnold, & Maldonado, 1995; Page, 2006). The extent of acculturation is directly related to the amount of time the individual has been exposed to the new culture, and inversely related to the strength of the previous cultural identity (Page, 2006).

The current study circumvents the above-mentioned limitations by considering East- and South-Asians as independent racial/ethnic groups and by controlling for factors such as height and weight, which are strongly related to bone density and body composition, and duration of stay in U.S. which may directly or indirectly influence bone mineral density and body composition, and other lifestyle factors such as calcium and vitamin D intakes and amount of physical activity. The unique findings from this study include that areal BMD at the left

femoral neck (FN) was higher in Caucasians compared to East-Asian women. Younger Caucasian women had a higher left FN areal BMD than younger South-Asian women, whereas, for the middle-aged group, these values were higher for both Caucasian and South-Asian women compared to East-Asians. For pQCT measured variables, total vBMD was higher in Caucasians than East- and South-Asian women, and stress-strain index (SSI) was higher in Caucasians compared to South-Asian women at 38% of the tibia. Moreover, endosteal circumference was higher in East-Asian than in Caucasian women, whereas, polar moment of inertia (IPOLAR) was higher in East-Asians compared to South-Asian women. At 66% of the tibia, total BMC was higher in East-Asian compared to South-Asian women, whereas, cortical BMC and area were higher in both Caucasian and East-Asian women compared to South-Asians. Middle-aged East-Asians also had greater periosteal and endosteal circumferences than Caucasians and South-Asians. Total body fat percentage and fat mass were significantly greater in South-Asian women than East-Asians and Caucasians, and A/G ratio was significantly higher in East-Asians compared to Caucasian women. Serum vitamin D levels were higher in Caucasians compared to both East- and South-Asians, while sun exposure scores were higher in Caucasians and East-Asians compared to South-Asians.

In addition to this, significant positive correlations were noted between age, height, weight, and areal BMD measures for Caucasians. For East-Asians, areal BMD was related to lean mass and muscle strength, while for South-Asians these relationships were significant for age, weight, tBPAQ, length of residency in U.S., jump test and body composition variables, and muscle density. Volumetric BMD, BMC and bone strength measures at 4% of tibia were positively related to height, weight, body composition variables, muscle strength and cross-sectional area, and negatively to age of menarche for Caucasians; to weight, BFLBM, and

muscle strength for East-Asians; and positively related to height, weight, body composition variables, muscle strength and physical activity, and negatively to age of menarche for South-Asians. For bone parameters assessed at 38% of tibia, the variables were significantly related to height, weight, body composition variables, muscle strength, and physical activity for Caucasians; to BFLBM, muscle strength and vitamin D intake for East-Asians; and to height, weight, body composition variables, muscle strength, and cross-sectional area, and contraceptive use for South-Asians. At 66% of tibia sites, vBMD, BMC and bone strength measures were positively related to height, weight, BFLBM, fat mass, and muscle strength for Caucasians; to BFLBM and muscle strength for East-Asians; and to height, weight, body composition, and muscle strength for South-Asians. Moreover, BFLBM, handgrip strength, and tBPAQ were significant predictors of lumbar spine and femoral neck areal BMD, and BSI and SSI for Caucasians and East-Asians, whereas, fat mass, BFLBM, and tBPAQ predicted these parameters in South-Asians.

Physical Activity, Calcium and Vitamin D Intakes

Physical activity is a beneficial health behavior that decreases the risk of chronic diseases like obesity, diabetes, cardiovascular disorders, and positively impacts bone mineral density (BMD) throughout life (Carter & Hinton, 2014). Peak BMD, which represents the maximal bone mass acquired by the end of skeletal maturity, is achieved by the end of the third decade of life (Heaney et al., 2000; Lu et al., 2016). Physical activity increases muscle mass and strength, thereby increasing the mechanical loads on the skeleton (Hughes & Petit, 2010). Therefore, physical activity is critical to increase bone mass in children and adolescents and augments the amount of peak bone mass gained in young adults. In middle-aged adults, its importance is reflected in attenuation in the rate of bone loss rather than an increase in bone

mass (Kohrt, Bloomfield, Little, Nelson, & Yingling, 2004). In the current sample, most of the participants had high (31.8%) to moderate (57.9%) levels of physical activity, and only 10.3% had low levels of physical activity (Table 9). The beneficial effects of physical activity on bone density can be demonstrated by the significantly positive relationships between physical activity scores and pQCT measured total and trabecular area, bone strength index, and total and cortical bone mineral content and area for both Caucasians and South-Asians in the current study. Moreover, total BPAQ scores were positively related to LS BMD for South-Asians and trabecular bone strength index (BSI) and vBMD at 4% of the tibia site for East-Asians. In addition to this, tBPAQ score was a significant predictor of LS BMD in South-Asians, and BSI and SSI in East-Asians (Table 31, 32).

The current study showed no significant differences in physical activity scores measured using International Physical Activity Questionnaire (IPAQ) and Bone Specific Physical Activity Questionnaire (BPAQ) for young and middle-aged premenopausal women (Table 8). These results were similar to those observed by Kim et al. (2018), who reported similar total BPAQ scores in young and middle-aged women for their sample. Additionally, Johannsen et al. (2008) and Laudani et al. (2013), reported no differences between physical activity levels assessed using wearable activity monitors in their younger and older groups. However, Bélanger, Townsend, & Foster, 2011, reported higher physical activity scores for their young group in comparison to the older participants. A reason for lack of age-related differences in the current study could be the narrow age ranges defining young and middle-age groups, 18-30 and >30-45 years, in contrast to Bélanger et al. who compared participants aged 18-24 years with those aged >65 years. Moreover, Johannsen et al. (2008) postulated that physical activity differences between young or middle-aged individuals and those aged >65 years are mostly due to lack of

occupation-related physical activity in the latter group, which constitutes the majority of the physical activity in young and middle-aged individuals.

Based on ethnicity, past and total BPAQ scores were significantly greater in Caucasians compared to East- and South-Asian women before controlling for duration of time the participants have stayed in the U.S. Following adjustment for covariates, these group differences were minimized, and the scores became similar across the three ethnic groups. Physical activity scores assessed using IPAQ were not different before or after controlling for covariates. Contrary to our results, previous studies have reported lower physical activity levels in Asians compared to Caucasians (Iliodromiti et al., 2016; Liang et al., 2007; Nightingale et al., 2016; Yi, Roberts, Lightstone, Shih, & Trinh-Shevrin, 2015). Yates et al. (2015), reported that although self-reported physical activity scores using IPAQ were higher in Caucasian men and women compared to South-Asians, objectively measured physical activity using activity monitors was similar in both the groups. However, a small difference was evident between women, where Caucasian women had higher objectively measured physical activity than South-Asian women. They concluded that cultural differences in the perception of what is considered as ‘moderate’ and ‘vigorous’ physical activity, and the fact that these questionnaires are developed for and validated in White populations may lead to an inflation of the results. Moreover, the diminishing group differences following adjustment for duration of time spent in U.S. signify that the previously existing differences between past and total BPAQ scores were mediated through differences in social and environmental factors related to the duration of time the participant has stayed in the U.S., like increased availability of fitness centers, social motivation, and opportunity to participate in group training exercise programs. The ease of access to these facilities can be linked to increasing urbanization and is more prevalent in

Western countries which results in greater participation in physical activity of participants who have spent more time in the U.S. in comparison to the immigrant population who are still getting accustomed to these changes (Ranasinghe et al., 2013). If these factors are controlled, as in the current study, physical activity remains similar across the three ethnic groups.

Along with physical activity, adequate calcium and vitamin D levels are other pertinent factors influencing BMD. Over 99% of the body's calcium is stored in the form of hydroxyapatite in bones and teeth, imparting strength and rigidity to the tissue. Intestinal absorption of dietary and supplemental calcium is dependent on calcitriol (1, 25- dihydroxy vitamin D) and primarily occurs in the duodenum where vitamin D receptors are expressed in their highest concentrations. Along with calcium, calcitriol also stimulates the absorption of phosphorus from the intestine. Thus, adequate calcium and vitamin D intake, through food sources, supplements, and exposure to sunlight, is critical to maintaining bone strength throughout life. The estimated average requirement and recommended dietary allowance for calcium and vitamin D for those aged 19-50 years is 800 and 1000 mg/day for calcium, and 400 and 600 IU/day for vitamin D respectively (Ross, 2010). However, there are other ongoing studies such as the VITAL trial (VITamin D and omega-3 trial) which is a prospective study assessing the benefits of higher vitamin D intakes, 2000 IU/day, on chronic diseases such as cancer, cardiovascular disorders and others (Feldman et al., 2013).

For the current study, Caucasians and South-Asians had higher calcium intake than East-Asian women. Although vitamin D intake was similar across age and ethnicity both before and after controlling for covariates, sun exposure scores were significantly greater in Caucasians and East-Asians than in South-Asians. Similar to this, serum vitamin D levels were significantly higher in Caucasians compared to East- and South-Asians (Table 8). Moreover, for participants

categorized as having low serum vitamin D levels, 43.6% were South-Asian, 30.8% were East-Asian, and only 25.6% were Caucasian (Table 10). Since naturally occurring food sources of vitamin D are scarce, cutaneously synthesized vitamin D remains the primary source of vitamin D production in humans. Lower sun exposure scores in South-Asians can be indicative of decreased cutaneous vitamin D synthesis, ultimately resulting in decreased serum vitamin D levels in comparison to Caucasian women (Feldman et al., 2013). These lower sun exposure scores can be attributed to decreased time spent doing outdoor activities such as leisure-time physical activity, or household activities like gardening, or walking for commute; increased clothing, which is considered culturally appropriate in South-Asian women and decreases the amount of exposed skin area for cutaneous synthesis of vitamin D; and a tendency to avoid sunlight to prevent tanning due to a social preference for a lighter skin color. In addition to this, the naturally high melanin levels in South-Asian women compared to Caucasians and East-Asians further restrict their cutaneous formation of vitamin D (Arya et al., 2004). However, the accuracy of the results of this questionnaire is limited due to its inability to account for factors like the amount and frequency of application of sunscreen which effectively decreases the capacity of the skin to synthesize vitamin D depending on the strength of its sun protection factor; season; time of day; cloud or tree cover; and even anatomical positioning of the body, sitting or standing, and the body site exposed (McCarty, 2008). Thus, even though South-Asians have higher calcium intake than East-Asian women, the unidirectional relationship between sun exposure scores and serum vitamin D levels can eventually result in decreased intestinal calcium absorption in South-Asians, decreasing their overall calcium levels. This also becomes a challenge for East-Asians who have lower serum vitamin D levels and low calcium intake. This increases the possibility of parathyroid hormone-mediated bone resorption in order

to bring the blood calcium levels back to normal (Feldman et al., 2013). However, while making such interpretations from these results it must not be forgotten that these questionnaires are subject to recall bias and personal preferences of the participants to adequately report their results and hence limited in their ability to quantitatively assess these factors.

Body Composition

Bone and muscle are located in close physical proximity and are mechanically and chemically interconnected to each other (Brotto & Johnson, 2014). Increased muscle mass increases the magnitude of mechanical stress on the bones inducing bone formation whereas stresses below the mechanical threshold result in bone resorption (Frost, 2000; Hirschfeld, Kinsella, & Duque, 2017). Thus, muscle mass is an important predictor of bone strength and can be quantified by DXA as a fat and bone free component, bone free lean body mass, which is largely constituted by muscle, along with some proportion of skin, tendons, and connective tissue (Silva et al., 2010). The positive relationship between muscle mass and bone is evident in the current study where bone free lean body mass is positively related to areal and volumetric BMD, BMC, and bone strength and stress-strain indices at all sites for the three ethnic groups. Bone free lean body mass is also a significant predictor of LS and FN areal BMD, and BSI and SSI for all the three ethnic groups (Tables 30-32). Additionally, bone free lean body mass (BFLBM) and appendicular skeletal muscle mass (ASM) were significantly higher in Caucasians compared to East-Asians. However, these differences disappeared following adjustment for height, weight, and duration of stay in U.S (Table 11).

In contrast to the current results, previous studies have shown that BFLBM and ASM are significantly higher in Caucasians in comparison to Asian women (Alekel et al., 1999; Liang et al., 2007; Morton et al., 2003; Sun et al., 2003). Alekel et al. (1999) reported greater bone free

lean mass in Caucasian women than in South-Asian Indian and Pakistani women, while, Liang et al. (2007) reported a higher lean mass in Caucasians compared to young Asian women. However, lean body mass is closely related to height and weight and differences in lean mass between populations can be reflective of differences in body mass and stature rather than true differences in amount of lean mass (Hume, 1966; Pomeroy, Macintosh, Wells, Cole, & Stock, 2018). Thus, it becomes necessary to statistically control for height and weight in order to determine actual differences in the amount of lean mass. This is critical in studies evaluating lean mass differences in different races/ethnicities as height and weight are known to vary across ethnicities (Sacker & Kelly, 2012). However, none of the above-mentioned studies adjusted for height and weight when evaluating lean mass differences which limit the accuracy of these results and make us wonder if these differences will exist after controlling for covariates.

Additionally, muscle functional capacity is impacted by proteins and low dietary protein intake is linked to loss of lean tissue, immune response and muscle function (Castaneda, Charnley, Evans, & Crim, 1995). Muscle protein synthesis has been shown to increase temporarily following protein or amino acid ingestion in both young and older women (Paddon-jones & Rasmussen, 2010). Previous studies have established an inverse relationship between dietary protein intake and length of residency in the U.S. in immigrant Asians (Talegawkar et al., 2016). Therefore, in addition to height and weight, this study also controlled for the duration of stay in the U.S. to decrease the influence of cultural differences in diet on body composition. It must be noted that these results represent a very stringent analyses which controls for anthropometric and cultural factors and demonstrates that after controlling these external factors there exist minimal differences, if any, in BFLBM between the three

racial/ethnic groups. Any differences remaining after controlling for these covariates can potentially be linked to genetic differences in skeletal muscle phenotype and metabolism between the three ethnic groups, however, this is beyond the scope of this study.

In spite of similar BFLBM values, percent body fat, and fat mass were higher in South-Asian compared to East-Asian and Caucasian women (Table 11). These results are supported by previous studies that have reported a higher percent body fat and fat mass in Asian Indian and Pakistani women compared to their Caucasian counterparts (Alekel et al., 1999; Chang et al., 2003; Kamath et al., 1999). Moreover, our results showed that fat mass and percent body fat were positively related to areal BMD at the total body and appendicular sites, and volumetric BMD and BMC at 4, 38 and 66% of the tibia for Caucasians and South-Asians. For East-Asians, this relationship was moderately negative for cortical vBMD at 38 and 66% of tibia sites. Additionally, fat mass was a significant predictor of FN BMD and BSI in South-Asians (Table 32).

A higher fat mass is linked to higher body weight, which is conventionally thought of as osteogenic due to the loading effects of increased body mass on the skeleton; association of fat mass with secretion of bone active hormones like insulin, amylin, preptin, and resistin from the pancreatic β -cells; and secretion of bone active factors such as leptin, estrogen, and adiponectin from the adipocytes (Ranasinghe et al., 2013; Rosen & Klibanski, 2009). On the other hand, excessive body weight has been associated with increased incidence of fractures at the radius in children and adolescents, indicating an anomaly in the conventional hypothesis, and suggesting that the positive relationship between bone and fat disappears or becomes negative once the increase in fat mass crosses a certain threshold, which remains unknown. Thus, the relationship

between bone and fat is complex and extends beyond the conventional mechanical interactions (Ilich et al., 2014).

Adipose tissue is metabolically active and releases pro-inflammatory cytokines such as TNF α , IL-6, and C-reactive protein, which create a condition of low-grade chronic inflammation. These proinflammatory cytokines can mediate osteoclastogenesis by upregulating the RANKL/RANK/OPG pathway and inducing bone resorption. Additionally, excessive adipose tissue may inhibit osteoblastogenesis by altering the fate of mesenchymal stem cells in the bone marrow niche by promoting adipogenesis (Duque, 2008; Hughes & Petit, 2010; Ormsbee et al., 2014). Finally, adipocyte-derived cytokines, leptin, and adiponectin, also influence bone metabolism. Increased leptin levels, as in obesity, negatively impact bone metabolism. Adiponectin inhibits osteoclastogenesis, however, its secretion decreases in obesity thus promoting osteoclastogenesis and increasing bone resorption (Barbour et al., 2014; Cao, 2008).

We also observed that A/G ratio was higher in East-Asians than in Caucasian women (Table 11). Chung et al. (2005), reported a lower fat mass in East-Asian women compared to Caucasians, however, waist-to-hip ratio was higher in East-Asians in comparison to Caucasian women. Morimoto et al. (2012) reported a higher trunk/peripheral fat ratio in Asians compared to White women. Although they did not find differences in A/G ratio, they did observe lower gynoid fat mass in Asians compared to White women confirming their hypothesis of higher central adiposity in Asians. They reported that these differences in fat distribution arise during childhood as their younger sample aged 10-16 years also had increased central adiposity in comparison to White girls. Our results of higher A/G ratio in East-Asian women were significantly different from Caucasian women even after controlling for height and weight

signifying that ethnic differences in fat distribution are independent of body size. Moreover, previous studies have demonstrated that Asians have an inherently low capacity to store fat in the superficial subcutaneous adipose tissue layer, implying that most of the excess energy is stored in either the deep subcutaneous fat layer or in visceral fat depots. This eventually results in ectopic fat deposition within tissues such as the skeletal muscle and liver (Wulan, Westerterp, & Plasqui, 2010). Abdominal fat can result in decreased bone formation by secreting cytokines which can decrease the amount of bone surface undergoing mineralization, decrease mineral apposition rates, and lower the number of activated remodeling units. Secondly, the association of abdominal fat mass with increased marrow fat can potentially modify the bone marrow milieu and result in greater adipogenesis in comparison to osteoblastogenesis (Cohen et al., 2013).

Areal Bone Mineral Density

Before adjusting for covariates, total body and lumbar spine BMD and BMC were higher in middle-aged than in younger women, and in Caucasians than in East- and South-Asians respectively. However, these differences disappeared after controlling for height, weight, and duration of time spent in the United States (Table 12). Contrary to this, previous studies have documented that LS BMD is higher in Caucasians than in Asian women (Davis et al., 1994; Marquez et al., 2001; Morton et al., 2003). Fielding et al. (2002) reported similar results in young women where LS BMD was significantly higher in Caucasians than in Asians before adjusting for covariates but became non-significant following adjustment for height and weight. These results were further supported by studies done by Danielson et al. (2013) and Walker et al. (2011; 2014), which reported similar LS BMD in pre-, peri-, and postmenopausal Caucasian and Asian women. Contrastingly, Liu et al. (2011) reported a higher LS BMD in their sample

of premenopausal Chinese-American women compared to White women after controlling for covariates. Similarly, Finkelstein et al. (2002) reported a higher LS BMD in perimenopausal Chinese and Japanese women in comparison to Caucasian women all of whom weighed less than 70 kg and were perimenopausal.

At the hip, left femoral neck BMD and BMC were higher in Caucasians than in East- and South-Asians and right femoral neck BMD was higher in Caucasians than in East-Asians. Trochanter and total hip BMD and BMC were both higher in Caucasians compared to East-Asians women. After controlling for covariates, these differences were significant only for the left FN BMD where Caucasians had higher values than East-Asians. Moreover, left FN BMD was significantly higher in young Caucasian women than in young South-Asians, and in middle-aged Caucasian and South-Asian women than in middle-aged East-Asians (Table 13).

Our results support previous literature where lower femoral neck and trochanter BMD is reported in Asians in comparison to Caucasian women (Alekel et al., 1999; Liang et al., 2007; Marquez et al., 2001; Nakamura et al., 1994; Silva, 2013). However, previous studies have also reported similar results for total hip and femoral neck BMD between Asian and Caucasian women (Walker et al., 2011; Danielson et al., 2013; Walker et al., 2014). In contrast to this, some studies indicate a higher total hip and femoral neck BMD in Asians compared to Caucasian women (Finkelstein et al., 2008; Khandewal et al., 2012; Walker et al., 2009). Finkelstein et al. (2002) showed that in perimenopausal women weighing less than 70 kg Chinese women had higher femoral neck bone mineral apparent density, a mathematically calculated substitute for volumetric BMD, than Caucasian women. Similarly, Khandewal et al. (2012), documented that in women aged 50-69 years FN BMD was higher in South-Asian and White women compared to Chinese women and higher in Whites than in South-Asians. This is

similar to our results in middle-aged women where Caucasians and South-Asians had higher FN BMD than East-Asian women. These contrasting results for areal BMD can be due to differences in sample characteristics, methodology, and in the model of DXA used for assessment of areal BMD. Additionally, the lack of differences at the total body, lumbar spine, trochanter, and total hip, can be due to the fact that our sample consists of premenopausal women within a narrow age range with a limited distribution for BMD. Future studies with a wide age range including peri- and post-menopausal women can help to identify the exact stage in life when these racial/ethnic differences in bone are most prominent. Unlike the LS BMD, BMD at the femoral neck is lower in East-Asians compared to Caucasians and middle-aged South-Asian women. This may indicate that ethnic differences in BMD are site-specific, where East-Asians are at a disadvantage at the appendicular but not at the axial sites.

Volumetric Bone Mineral Density

Our results demonstrate a higher trabecular vBMD at 4% of the tibia in younger East-Asians compared to young South-Asians. Total BMC, and cortical BMC and area at 66% of tibia sites were higher in East-Asians and Caucasians compared to South-Asian women. Total vBMD at 38% of the tibia was greater in Caucasians compared to East- and South-Asians. Moreover, SSI was greater for Caucasians and IPOLAR was greater for East-Asians in comparison to South-Asian women at 38% of the tibia. East-Asians also had greater periosteal and endosteal circumferences at 38% and 66% of the tibia compared to South-Asians (Tables 14-16). Volumetric bone mineral density (vBMD) is related to the degree of calcification and stiffness of the bone and is representative of material properties of the bone tissue, whereas IPOLAR and SSI are indicative of structural stiffness of the bone to torsional bending (COUNTRY et al., 2014; VLOK et al., 2019).

Our results are supported by studies performed by Walker et al. (2011) using high-resolution pQCT to assess bone microarchitecture in Chinese-American and Caucasian women. They concluded that the Chinese skeleton has mechanical advantages both at the tibia and radius, thereby offering greater resistance to fracture loads and possibly explaining their lower fracture rates in spite of a similar or lower areal BMD at the appendicular sites in comparison to Caucasian women. They reported a higher total, cortical and trabecular volumetric BMD and thickness at the tibia and radius in Chinese American than in Caucasian women (Walker et al., 2009). At the tibia, trabecular number was higher for Caucasian than Chinese American women, while trabecular thickness was higher for Chinese American women (Walker et al., 2011; Walker et al., 2013). In addition to this, higher cortical porosity has been reported in Caucasian than in Chinese American women both at the tibia and radius (Boutroy et al., 2014). Using micro-finite element analysis based computer modeling technique, Liu et al. (2011), simulated the resistance offered by the bone tissue to fracture loads and concluded that Chinese-American women had higher resistance to loads in mediolateral and longitudinal directions both at the radius and tibia compared to Caucasian women. We were unable to track similar studies including South-Asian women. Although these studies utilize high-resolution pQCT to assess bone microarchitecture, our pQCT derived bone parameters are similar to these results and signify better material and structural bone properties in East-Asians in comparison to South-Asian women at the tibia.

Moreover, East-Asian women in the current study had greater periosteal and endosteal circumference at 66% tibia than Caucasian and South-Asian women. The deposition of bone mass over a greater cross-sectional area (CSA) results in greater resistance to bending forces than the same amount of bone in a smaller CSA as resistance to bending is proportional to the

fourth power of distance from the neutral axis (Seeman, 2008). Therefore, having a larger periosteal circumference provides geometric benefits to East-Asians against bending forces. This can also potentially attenuate the menopause-related bone loss which primarily occurs due to decreased periosteal apposition and increased endocortical resorption, thus thinning the cortical bone. But since in East-Asians the bone mass is distributed around a larger perimeter it would still offer greater resistance to bending forces than if the same bone mass was distributed around a smaller cross-sectional area as in the case of Caucasians. Additionally, South-Asians have a lower BMC and a similar or lower vBMD than East-Asians and Caucasians. A decreased bone mass along with a decreased periosteal circumference disadvantages this population both materially and geometrically (Szulc, Seeman, Duboeuf, Sornay-Rendu, & Delmas, 2006). These differences, as we see cannot be detected by DXA assessed areal BMD which shows no differences at the total body and lumbar spine, and a lower BMD at the femoral neck for East-Asians. Thus, similar to the above-mentioned studies, superior material and geometric properties at the tibia in East-Asians potentially provide an explanation for their lower fracture rates at the appendicular skeleton in spite of lower areal FN BMD.

Muscle Function Assessment

After controlling for covariates, our results show that 1-RM leg press strength was significantly higher for Caucasians compared to South-Asian women. Additionally, jump height and time were higher in younger in comparison to middle-aged women (Table 17). Moreover, handgrip strength was a significant predictor of SSI at 38% of the tibia for Caucasians, and LS BMD and SSI at 66% of the tibia for East-Asians (Tables 30, 31). The jump test allows assessment of the neuromusculoskeletal system and has the potential to quantify an individual's bone and tendon stiffness and elasticity, balance and muscle function

(Buehring, Krueger, & Binkley, 2010; Singh et al., 2014). Our results for jump test are similar to those of Buehring et al. (2010), who reported a greater jump height in young groups in comparison to older individuals.

Leg muscle strength measured using 1-RM leg press test is a strong predictor of lower limb BMD and is reported to be highest in Caucasians, followed by Hispanics, and least in Asians (Liang et al., 2007). Moreover, Davis et al. (1999), reported a higher triceps and quadriceps muscle strength in Caucasians in comparison to Asian women. Skeletal muscle properties and physical performance vary with race/ethnicity, however, studies comparing these differences are limited and mostly include Caucasian and African-American populations (Araujo et al., 2010; Rantanen et al., 1998; Suminski et al., 2002). Studies including Asians are scarce, with no traceable studies which included South-Asians. Thus, there is a critical need for future studies quantifying racial/ethnic differences in muscle strength using handgrip strength, jump test, dynamometry, and electromyography to provide a more accurate understanding of the underlying mechanisms regulating racial/ethnic differences in bone health.

CHAPTER V

CONCLUSIONS

The primary purpose of this study was to determine differences in bone mineral density, bone free lean body mass and muscle strength, and fat mass, in premenopausal women aged 18-45 years belonging to three different racial/ethnic groups: Caucasians, South-Asians (SA), East-Asians (EA). For each ethnicity, the given age range (18-45 years) was divided into two sub-groups: 18-30 years (young), and >30 to 45 years (middle-aged), to allow comparison between women who are accruing bone mass vs. those who have achieved their peak bone mass.

The following research questions were investigated:

Research Question 1: Is there a significant difference in bone status, areal and volumetric BMD, in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

Yes, significant ethnicity and age-related differences were observed for areal BMD at the left femoral neck, and total vBMD, SSI, IPOLAR and endosteal circumference at 38% of the tibia, and total BMC, and cortical BMC and area at 66% of the tibia.

After controlling for covariates, left femoral neck areal BMD was significantly higher in Caucasians in comparison to East-Asian women. There were no significant differences for total body, lumbar spine, trochanter, and total hip BMD based on age or ethnicity.

There were no significant main effects of ethnicity or age at 4% of the tibia site, however, total vBMD was higher in Caucasians than East- and South-Asian women at 38% of the tibia. Additionally, SSI was higher in Caucasians compared to South-Asian women, while IPOLAR was higher in East-Asians compared to South-Asian women. Endosteal circumference was higher in East-Asian than in Caucasian women.

At 66% of the tibia site, total BMC was higher in East-Asian compared to South-Asian women, and cortical BMC was higher in both Caucasian and East-Asian women compared to South-Asians. The cortical area was significantly greater in Caucasians in contrast to both East- and South-Asians. Based on age, muscle density was greater in younger than in middle-aged women.

Research Question 2: Is there a significant difference in body composition parameters, BFLBM, and muscle strength, and fat mass, in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

Yes, body composition and muscle strength were significantly different based on ethnicity and age. Total body fat percentage and fat mass were significantly greater in South-Asian women than East-Asians and Caucasians, and android/gynoid ratio was higher in East-Asians compared to Caucasian women. For muscle strength, 1-RM leg press strength was significantly greater in Caucasian compared to South-Asian women. Based on age, jump height and time were higher in younger in comparison to middle-aged women.

Research Question 3: Is there a significant interaction between age groups (young vs. middle-aged) and race/ethnicity (Caucasians, EA, SA), for bone status (areal and volumetric BMD) and body composition parameters (BFLBM and strength, fat mass)?

Yes, significant Ethnicity X Age interactions were observed for left FN aBMD, trabecular vBMD at 4%, and periosteal and endosteal circumference at 66% of tibia sites. Younger Caucasian women had a higher left FN aBMD than younger South-Asian women, whereas for the middle-aged group these values were higher for both Caucasian and South-Asian women compared to East-Asians. Trabecular vBMD at 4% of tibia was higher in young East-Asians compared to young South-Asians, and within East-Asians, were higher for young

than middle-aged women. At 66% of tibia, periosteal circumference was significantly greater in middle-aged East-Asians compared to middle-aged Caucasians and South-Asians, and endosteal circumference was greater in middle-aged East-Asians compared to middle-aged Caucasian women.

The following research sub-questions were investigated:

Research Sub-Question 1: Is there a significant difference in circulating vitamin D levels in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

Yes, serum vitamin D levels were significantly higher in Caucasians compared to both East- and South-Asians, while sun exposure scores were higher for both Caucasians and East-Asians compared to South-Asians. Vitamin D intake was similar across the three ethnicities. There were no age-related differences in serum vitamin D levels, vitamin D intake or sun exposure scores.

Research Sub-Question 2: Is there a significant difference in physical activity (PA) levels in premenopausal women aged 18-45 years (young vs. middle-aged) belonging to different ethnicities- Caucasians, EA, and SA?

After controlling for the duration of residency in United States, there were no significant differences for physical activity across ethnicity or age.

Our central hypothesis for this study was that South-Asian women will have a higher fat mass and lower bone free lean body mass (BFLBM) and muscle strength in comparison to East-Asians and Caucasians, a phenotype that expedites bone loss and helps to explain the early occurrence of osteoporotic fractures in this population. This hypothesis was based on our pilot study which assessed bone mineral density, BFLBM, fat mass, and muscle strength in women

aged 18-30 years from Caucasian, East-Asian, South-Asian, Hispanic and African-American backgrounds. Forty-one participants were included in this study. This study concluded that South-Asian women have a higher fat mass and percent body fat, and lower BFLBM and muscle strength than East-Asians and Caucasians. Additionally, physical activity was significantly lower in South-Asians in comparison to East-Asians and Caucasians.

For the current study, we found that South-Asians had a higher fat mass and percent body fat than Caucasians and East-Asians, and a lower leg muscle strength than Caucasians. Moreover, left femoral neck areal BMD was lower in young South-Asian women compared to young Caucasians. South-Asians also had decreased trabecular vBMD at 4% and reduced total and cortical BMC at 66% of the tibia. Additionally, South-Asian women had significantly lower SSI, IPOLAR, cortical area, and periosteal and endosteal circumferences at 38% and 66% of the tibia, in comparison to East-Asians and Caucasians. A higher fat mass has the potential to accelerate bone loss as adipocytes secrete proinflammatory cytokines which can promote osteoclastogenesis and also interfere with mesenchymal stem cell differentiation, promoting adipogenesis instead of osteoblastogenesis. Additionally, the lower femoral neck BMD values in young South-Asian women can be attributed to their inability to gain adequate bone mass at skeletal maturity. The reasons for this can be manifold and can range from the genetic propensity of South-Asians for a low bone mass to inadequate non-genetic factors, like low serum vitamin D levels and sun exposure, and decreased muscle strength, as evident by the results of this study. The amount of peak bone mass gained at skeletal maturity is directly related to the amount of bone mass in adulthood (Lu et al., 2016). As South-Asians are at the lower end of the population distribution at a young age when peak bone mass is being achieved, they will likely be at the lower end for BMD even during later adulthood. Thus, a low bone

mineral density, along with an incompetent bone structure, increased fat mass, and decreased muscle strength can potentially help to explain the early incidence of osteoporotic fractures in this population.

Our results also demonstrate that left femoral neck BMD was lower in East-Asians compared to Caucasians women. However, unlike South-Asians, in spite of a lower femoral neck areal BMD, East-Asians had a higher trabecular vBMD at 4% of the tibia, and higher IPOLAR and total and cortical BMC at 38% and 66% of the tibia, in comparison to South-Asians. Additionally, periosteal and endosteal circumferences at 66% of the tibia were higher in East-Asians compared to Caucasians and South-Asians. The higher android/gynoid ratio in East-Asians is indicative of greater abdominal adiposity which can result in decreased BMD as adipocyte-secreted factors can result in increased bone resorption and are also linked to inhibition of bone marrow osteoblastogenesis. However, in spite of a lower areal BMD, a highly preserved bone structure in East-Asians, evident by their higher trabecular vBMD, BMC, IPOLAR, and periosteal and endosteal circumferences, offers greater resistance to fracture loads and helps to explain the lower fracture incidence in this population, particularly at the appendicular sites.

We did not find any differences in physical activity or BFLBM for the current sample, which is contrary to what we hypothesized based on our pilot data. One of the reasons for this could be the small sample size of the pilot study, which limited our ability to control for confounding variables and impacted the interpretability of those results.

Clinical Significance

Osteoporosis remains a public health problem in all racial/ethnic groups, particularly in Asians resulting in their rapidly increasing fracture rates. The current study evaluates the bone-muscle-fat unit concurrently, by keeping bone in the forefront, in Caucasian, East-Asian, and South-Asian premenopausal women in the age range of 18-45 years. This age range is further subdivided into two groups: young (18-30 years); middle-aged (>30-45 years), to allow comparison between women who are accruing bone mass compared to those who have achieved their peak bone density. For young women, this can provide an opportunity to optimize their peak bone density by improving their nutrition and physical activity levels, leading to decreased vulnerability to future fractures. Appropriate exercises can include high impact activities like jumping, gymnastics, basketball, which are known to be osteogenic and introduce loads in multiple directions. For middle-aged premenopausal women, an accelerated bone loss due to menopause is impending. Appropriate exercises can include moderate loading activities and resistance training. Thus, assessment of bone health and efforts to maintain the same by increasing muscle strength, or decreasing body fat, or both, are paramount, so that a clinical diagnosis of osteoporosis can at least be delayed.

The results of this study highlight that “*one size does not fit all*” as there are racial/ethnic differences in these tissues even after controlling for body size and length of residency in the United States. Investigating the relationships between these tissues is necessary for the development of effective exercise protocols, therapeutic and preventative strategies to improve musculoskeletal health and decrease the physical and economic burden of musculoskeletal disorders like osteoporosis, particularly in high-risk minority populations. Moreover, the results of this study can be used for creating awareness among the at-risk

ethnicities regarding the importance of adequate physical activity and dietary practices in enhancing bone density.

Future Directions

As mentioned previously, future studies including larger sample sizes and evaluating these factors in peri- and post-menopausal women should be designed. Dual-energy X-ray absorptiometry is the current gold standard for the assessment of BMD and fracture risk. However, biomechanical principles indicate that fracture occurs when the load applied to a bone exceeds bone strength, which is dependent on both density and structure of the bone. Thus, along with BMD, microstructural properties of the cortical and trabecular compartments of the bone contribute significantly towards bone strength. This creates a need for future studies using techniques such as high resolution peripheral quantitative computed tomography (HR-pQCT) coupled with computer-based finite element analysis (FEA) modeling to examine bone microarchitecture and to estimate bone strength and load distribution, especially during menopausal transition period, a critical time for bone loss across all racial groups. Moreover, studies focusing on other Asian sub-groups within and across continents are required to understand the reasons behind the increasing fracture rates within the Asian ethnicity.

Our results also emphasize the need for future longitudinal investigations focusing on the effectiveness of different exercise programs (endurance, resistance training, whole-body vibration) to increase BMD and prevent fracture risk in these populations. Along with race/ethnicity, these exercise programs should focus on the age of the participant, i.e. whether the participant is premenopausal and bone accrual is still occurring or bone accrual is complete, peri-menopausal, or post-menopausal.

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APPENDICES

Appendix A: Informed Consent and HIPAA

701A Consent Version:

IRB Number: 9314

Consent Form

University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma (OU) – Norman Campus

Racial/Ethnic Differences in Bone Status, Muscle Function, and Fat Mass, in Young and Middle-Aged Premenopausal Women belonging to Caucasian, South-Asian, and East-Asian Backgrounds

Sponsor: OU Department of Health & Exercise Science
Principal Investigator: Michael Bemben, PhD
405-325-2717

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?

You are being asked to take part in this study because you are a woman, in the age range of 18-45 years old and either Caucasian, East-Asian, or South-Asian.

Why Is This Study Being Done?

The main purpose of this study is to examine differences in bone status, muscle function, and fat mass, in young and middle-aged premenopausal women belonging to different racial/ethnic groups.

How Many People Will Take Part In The Study?

About 150 women will take part in this study.

What Is Involved In The Study?

If you take part in this study, three visits will be needed.

The first visit consists of consent, questionnaires, blood pressure, and familiarization of the muscle strength and power tests which will take approximately 1.5 hours.

- Informed consent and Health Information Privacy form (HIPAA) - you must sign and date an informed consent form (this document) and HIPAA form stating that you understand all procedures and your rights as a participant.
- Health Status Questionnaire, Physical Activity Readiness Questionnaire, and menstrual history questionnaire - you may be excluded from the study if any answer on these questionnaires indicates that you are not eligible for this study.
- International Physical Activity Questionnaire and Bone Physical Activity Questionnaire - These questionnaires will assess your past and current physical activity levels
- Calcium intake questionnaire – This questionnaire will estimate how much calcium you take in by your diet and supplements.
- Food frequency questionnaire - This questionnaire will be used to record a three-day diet history (2 weekdays, 1 weekend day) to estimate the average daily calorie consumption, and carbohydrates, proteins, fats, calcium and vitamin D intake.

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IRB NUMBER: 9314
IRB APPROVAL DATE: 05/06/2019
IRB EXPIRATION DATE: 04/30/2020

- Sun exposure questionnaire - This questionnaire helps to assess participant's sunlight exposure based on the average time spent outdoors and the amount of skin exposed. This determines the role of cutaneous vitamin D synthesis in vitamin D status.
- Ethnicity identification form - This form will record information regarding the race/ethnicity of the participant; race/ethnicity of the biological parents, and grandparents; country of birth; number of years lived in U.S.
- Blood pressure measurement – you will be measured at least twice to determine if you are eligible for this study or not based on blood pressure.
- Familiarization of Muscle and Power tests - trained personnel will instruct you on correct techniques for the hand-grip, the two-leg press, and the vertical jump. After the instructions, participants will practice at light intensities becoming accustomed to the movements.

The second visit consists of a venipuncture blood draw.

Venipuncture blood draw (about 7.5 ml) (~20 minutes) The blood draw will be performed by a registered nurse or phlebotomist at the OU Goddard Student Health Center in the early morning after an overnight fast to measure serum levels of sclerostin, vitamin D, adiponectin, and Follicle stimulating hormone for women ≥ 40 years.

The third visit will consist of measuring your height and weight, your bone mineral density, lower body muscle strength and power, and handgrip strength and will take about 1.5 hours.

- Body weight and height will be measured (approximately 2 minutes)
- Urine Pregnancy Test (approximately 10 minutes) - This will be performed to ensure that none of the women are pregnant prior to initiating any radiation scans.
- DXA scans (approximately 20 minutes) – DXA scans will be used to determine the bone mineral density of the total body, lumbar spine, and dual proximal femur, and total and regional body composition. These tests are non-invasive. You will be lying on your back on the DXA table for the scans and you will be required to remain still during the procedures. DXA is a radiation procedure and is for research purposes only. There are risks associated with DXA which will be addressed below.
- pQCT scans (approximately 20 minutes) –The pQCT scan is a simple, non-invasive procedure during which you will sit as still as possible in a chair with your non-dominant leg in a leg support. The pQCT scan is a radiation procedure and is for research purposes only. There are risks associated with pQCT which will be addressed below.
- Handgrip Test (~10 min) – will be measured three times for both hands with a handgrip device. You will sit on a chair with back and measured forearm supported. You will be encouraged to squeeze as hard as possible and maximum grip strength for each hand in each device will be recorded.
- Jump Test (~10 min) – will be measured three times on a jump mat with power and speed analyzer. You will be asked to do a countermovement vertical jump by crouching, then jumping with non-restricted arm motion, and then landing on the jump mat. Trained spotters will be standing on your side to help with balance, if needed. A transfer belt will



be fastened around your waist to be held by the spotter to stabilize you if you lose your balance.

- Two Leg Press (approximately 15 minute) - Two leg muscle strength will be assessed by a standard 1- Repetition Maximum Test (1- RM). You will be semi- reclined on a leg press machine and will complete approximately 5- 6 repetitions with a load approximately equal to 50% of your estimated maximal strength. Following a 1 minute rest, you will then complete 3- 4 repetitions with a load approximately equal to 75% of your estimated maximal strength. Then following a 2 minutes rest loads will be increased so that a maximal voluntary effort is achieved within 5 more attempts. Each attempt during this section of the test will be separated by about 2- 4 minutes of rest.

How Long Will I Be In The Study?

We think that you will be in the study for 3 visits lasting for a total of about 3.5 hours.

There may be unanticipated circumstances under which your participation may be terminated by the investigator without regard to your consent; for example, your participation may be terminated if you become pregnant.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher first.

What Are The Risks of The Study?

While on the study, you are at risk for these side effects. However, there may also be unforeseeable risks with participation. You should discuss these with the researcher prior to providing your consent.

1) Risks and side effects related to having DXA and pQCT scans:

This study involves radiation exposure from 4 DXA scans and 3 pQCT scans which are types of x-ray procedures. These procedures are for research only and not needed for your medical care. The amount of additional radiation to which you will be exposed is approximately 1% of the amount of radiation to which we are exposed annually from background sources such as the Earth and Sun. In addition to any radiographic procedures performed as part of this research, you may also be exposed to radiation from procedures that are part of your normal care. The risk from radiation exposure increases over your lifetime as you receive additional exposure to radiation.

2) Risks and side effects related to functional performance tests:

There is a slight possibility of mild soreness due to muscle strength and power testing. Additionally, there is a slight risk of injury/fall during jump test.

3) Risks for blood draw:

There may be temporary discomfort, pain, and bruising at the site of the blood draw. You may feel faint and have a slight risk of infection.

4) Reproductive risks:



You must not be and should not become pregnant nor breast-feed an infant while on this study. Participating in the bone scans (DXA scans) involved in the study while you are pregnant, or breastfeeding may involve risks to an embryo, fetus, or infant, including birth defects which are currently unforeseeable. In order to reduce your risk of pregnancy, you or your partner should use one or more of the acceptable methods of birth control listed below, regularly and consistently, while you are in this study. Acceptable methods of birth control (continuing throughout the study) include:

- An approved oral contraceptive (birth control pill)
- Intra-uterine device (IUD)
- Hormone implants or Contraceptive injection (Depo-Provera)
- Barrier methods (diaphragm with spermicidal gel or condoms)
- Transdermal contraceptives (birth control patch)
- Vaginal contraception ring (birth control ring)
- Sterilization (tubal ligation, hysterectomy or vasectomy)

If you become pregnant or suspect that you are pregnant during this study, you should immediately inform the study personnel. If you become pregnant or suspect that you are pregnant while on this study a pregnancy test will be done. If pregnancy is confirmed, you may be withdrawn from the study.

Are There Benefits to Taking Part in The Study?

There are no direct benefits from participating in this study.

What Other Options Are There?

Your alternative is to not participate.

What About Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations outside the OUHSC that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration and other regulatory agencies and the OU Department of Health & Exercise Science. , The OUHSC Human Research Participant Program office, the OUHSC Institutional Review Board, and the OUHSC Office of Compliance may also inspect and/or copy your research records for these purposes.

What Are the Costs?

There is no cost to you for participating in this study.

Will I Be Paid For Participating in This Study?

There will be no compensation for participation in this study.



What if I am Injured or Become Ill While Participating in this Study?

In the case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company may be expected to pay the usual charge for this treatment. No funds have been set aside by The University of Oklahoma or The University of Oklahoma Health Sciences Center to compensate you in the event of injury.

What Are My Rights As a Participant?

Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, please be sure to discuss leaving the study with the principal investigator. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?

If you have questions, concerns, or complaints about the study or have a research-related injury, contact Dr. Michael Bemben at 405-325-5211 or mgbemben@ou.edu.

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Future Communications

The researcher would like to contact you again to recruit you into future studies or to gather additional information.

_____ I give my permission for the researcher to contact me in the future.

_____ I do not wish to be contacted by the researcher again.



Signature:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18) Printed Name Date

SIGNATURE OF PERSON
OBTAINING CONSENT Printed Name Date



University of Oklahoma Health Sciences Center Research Privacy Form 1
PHI Research Authorization

**AUTHORIZATION TO USE or SHARE
HEALTH INFORMATION THAT IDENTIFIES YOU FOR RESEARCH**
*An Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.*

Title of Research Project: **Racial/ethnic differences in bone status, muscle function, and fat mass, in young and middle-aged premenopausal women belonging to Caucasian, South-Asian, and East-Asian backgrounds**

Leader of Research Team: **Michael G Bembien, PhD**

Address: **1401 Asp Ave., Room 104, Norman, OK 73019**

Phone Number: **405-325-2717**

If you decide to sign this document, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and results of the following: ethnicity identification form, calcium intake questionnaire, food frequency questionnaire, health status questionnaire, menstrual history questionnaire, physical activity readiness questionnaire (PAR-Q), bone specific physical activity questionnaire (BPAQ), sun exposure questionnaire, and international physical activity questionnaire (IPAQ), blood pressure, blood draws (sclerostin, vitamin D, adiponectin); DXA scans (total body, lumbar spine, dual proimal femur), pOCT (at 4%, 38% and 66% of the non-dominant tibia length), hand grip test, jump test, two leg press test.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to determine racial/ethnic differences in bone status, muscle function, and fat mass, in young and middle-aged premenopausal women (18-45 years) belonging to Caucasian, South-Asian, and East-Asian backgrounds.

¹ Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.

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**University of Oklahoma Health Sciences Center Research Privacy Form 1
PHI Research Authorization**

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with no one else.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OUHSC.

Canceling Permission. If you give the OUHSC researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OUHSC researchers to use or share your PHI for their research will never end.

Contacting OUHSC: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official	or	Privacy Board
University of Oklahoma Health Sciences Center		University of Oklahoma Health Sciences Center
PO Box 26901		PO Box 26901
Oklahoma City, OK 73190		Oklahoma City, OK 73190

If you have questions, call: (405) 271-2511 or (405) 271-2045.

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical

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**University of Oklahoma Health Sciences Center Research Privacy Form 1
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information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

Patient/Participant Name (Print): _____

Signature of Patient-Participant
or Parent if Participant is a minor

Date

Or

Signature of Legal Representative**

Date

**If signed by a Legal Representative of the Patient-Participant, provide a description of the relationship to the Patient-Participant and the authority to act as Legal Representative:

OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

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Appendix B: Questionnaires

Screening Checklist

Racial/Ethnic Differences in Bone Status, Muscle Function, and Fat Mass, in Young and Middle-Aged Premenopausal Women Belonging to Caucasian, South-Asian, and East-Asian Backgrounds

Name: _____ Date: ____/____/____

Does subject meet the inclusion criteria for the study?	YES	NO
• Female	_____	_____
• Age 18-45 years	_____	_____
• Do you identify yourself as a Caucasian, East-Asian or South-Asian?	_____	_____

Does subject have any exclusion criteria for the study?	YES	NO
• Any disabilities	_____	_____
• Uncontrolled hypertension (140/ 90)	_____	_____
• Weight over 300 lbs.	_____	_____
• Height over 6 feet	_____	_____
• Joint replacement / metal implants at hip or spine	_____	_____
• Are you pregnant or think you may be pregnant	_____	_____

Qualify for the Study? _____

Bone Density Research Laboratory
OU Department of Health and Exercise Science
Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.
(NOTE: The following codes are for office use only: RF; MC)

Part 1. Information about the individual

1. _____

Date

2. _____

Legal name

Nickname

3. _____

Mailing address

Home phone

Business/cell phone

4. Gender (circle one): Female Male (RF)

5. Year of birth: _____ Age _____

6. Number of hours worked per week:

NA (retired) Less than 20 20-40 41-80 Over 80

If not retired, more than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8. Date of: Last medical physical exam: _____ Last physical fitness test: _____

Year

Year



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9. Circle operations you have had:

Back Heart (MC) Kidney Eyes Joint Neck

Ears Hernia Lung Other _____

NONE

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism	Diabetes	Kidney problem (MC)
Anemia, sickle cell	Emphysema	Mental illness
Anemia, other	Epilepsy	Neck strain
Asthma	Eye problems	Obesity (RF)
Back strain	Gout	Osteoporosis
Bleeding trait	Hearing loss	Phlebitis (MC)
Bronchitis, chronic	Heart problems	Rheumatoid arthritis
Cancer	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia	Thyroid problem
Concussion (MC)	Hyperlipidemia (RF)	Ulcer
Congenital defect	Infectious mononucleosis (MC)	Other _____

NONE

11. Circle all medicine taken in last 6 months:

Asthma (list type) _____	High-blood-pressure medication (list type) _____
Blood thinner (MC)	Epilepsy medication
Corticosteroids	Estrogen
Depression	Heart-rhythm medication (MC)
Diabetic pill	Insulin (MC)
Digitalis (MC)	Nitroglycerin (MC)
Diuretic (MC)	

Thyroid _____
Other _____

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood (MC)	d. Leg pain (MC)	g. Swollen joints (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
b. Abdominal pain (MC)	e. Arm or shoulder pain (MC)	h. Feel faint (MC)
1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
c. Low back pain (SLA)	f. Chest pain (RF) (MC)	i. Dizziness (MC)



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1 2 3 4 5

1 2 3 4 5

1 2 3 4 5

j. Breathless with slight exertion (MC)

1 2 3 4 5

Part 3. Health-related behavior

13. (RF) Do you now smoke? Yes No

14. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled			Less than 5, none inhaled

15. Weight now: _____ lb. One year ago: _____ lb. Age 21: _____ lb.

16. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

17. Do you exercise or labor at least three times a week?

1. Yes 2. No



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Subject ID _____

Date ____/____/____

ETHNICITY IDENTIFICATION FORM

- Please select the racial category with which you most closely identify by placing a check in the appropriate box.

- Caucasian
- East-Asian
- South-Asian

- Please select the racial category to which your biological parents most closely identify by placing a check in the appropriate box.

MOTHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological mother

FATHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological father

- Please select the racial category to which your biological grand-parents most closely identify by placing a check in the appropriate box.

MATERNAL GRAND-MOTHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological maternal grand-mother



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Subject ID _____

Date ____/____/____

MATERNAL GRAND-FATHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological maternal grand-father

PATERNAL GRAND-MOTHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological paternal grand-mother

PATERNAL GRAND-FATHER

- Caucasian
- East-Asian
- South-Asian
- I do not know the racial category of my biological paternal grand-father

- Your country of birth _____

If your country of birth is not United States:

- Number of months/years lived in United States _____



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Subject ID: _____ Date: _____

**Bone Density Research Laboratory
Department of Health and Exercise Science
University of Oklahoma**

MENSTRUAL HISTORY QUESTIONNAIRE

We are asking you to give us as complete a menstrual history as possible. All information is strictly confidential.

Are you pregnant (circle your response)

YES- Do not complete the rest of this form

NO- Continue to section A.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?
(please circle what months you have had a period. This means from this time last year to the present month)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

2. What is the usual length of your menstrual cycle (first day of your period to the next onset of your period)?

_____ days. Today is day _____ of your present menstrual cycle.

3. What was the date of the onset of your last period?

4. When do you expect you next period?

5. What is the average length (number of days) of your menstrual flow? _____ days

How many of these days do you consider "heavy"? _____ days

6. Do you experience cramps during menstruation (dysmenorrhea)? If yes, how many days does this last?

7. Do you experience symptoms of premenstrual syndrome (i.e., weight gain, increased eating, depression, headaches, anxiety, breast tenderness)? If yes, please list the symptoms.



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8. Do you take oral contraceptives or any other medication that includes estrogen and/or progesterone?

If yes, how long have you been taking this medication? _____

What is the brand name and dosage of this medication? _____

Has this medication affected your menstrual cycle (regularity, length and amount of flow)? If yes, indicate changes.

9. Have you taken oral contraceptives in the past? If no, skip to SECTION B.

If yes, what was the brand name and dosage? _____

When did you start taking the pill; for how long; and when did you stop taking it?

10. If you answered yes to 9 or 10, did you experience a weight gain and/or a change in appetite as a result of oral contraceptive use? If so, please indicate amount of weight gained. _____ lbs

SECTION B: PAST MENSTRUAL HISTORY

1. At what age did you experience your first menstrual period?

2. Were your periods regular (occurring monthly) during the first two years after menstruation began? If not, at what age did your period become regular?

3. Has there been any time in the past where your periods were irregular or absent? If no, skip to question 4. If yes, did these periods coincide with unusual bouts of training, or with a period of stress?

4. If you have had an irregular period due to training please describe (i.e., you have a period in the offseason but only irregular menstruation during preseason and season)?

5. Have you ever consulted a doctor about menstrual problems (specifically, about irregular or missing periods)? If no, skip to question 6.

Have you ever been diagnosed as having a shortened luteal phase (the time in between periods)?

6. Have you ever consulted a doctor about any problems relating to your hormonal system? If so, please explain.



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**BONE DENSITY RESEARCH LABORATORY
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
UNIVERSITY OF OKLAHOMA**

CALCIUM INTAKE ESTIMATION

Subject ID: _____ TODAY'S DATE: _____

Complete this form (where indicated) to represent your dietary intake in the past year.

Tally <i>(office use only)</i>	Score <i>(office use only)</i>	Food Type	serving size	I EAT THIS FOOD:	
				EVERY WEEK	EVERY DAY
				write in # servings/week	write in # servings/day
	300	Milk- whole, 2%, skim	1 cup		
	150	Cheese food or spread	1 oz		
	150	Cheese sauce	1/4 cup		
	150	American cheese	1 slice		
	150	Cottage cheese	1 cup		
	250	Ricotta cheese	1 oz		
	150	Blue cheese	1/2 cup		
	200	Natural cheese (except cream cheese) includes cheddar, Swiss, mozzarella, and so forth	1 oz		
	285	Buttermilk	1 cup		
	300	Yogurt, flavored or plain	1 cup		
	450	Fast Food Milkshake	12 oz		
	165	Cocoa from mix	1 packet		
	330	Eggnog	1 cup		
	280	Chocolate milk	1 cup		
	250	Macaroni and cheese, cheese souffle, lasagna, quiche, cannelloni, pizza	1 serving		
	180	Cream soup or chowder with milk	1 cup		
	115	Almonds	1/3 cup		
	180	Broccoli	1 cup		
	85	Beet greens, spinach	1/2 cup		
	160	Baked beans	1 cup		
	100	Figs	5 dried		
	140	Scalloped potatoes	1 cup		
	150	Soybeans	1 cup		
	150	Tofu	1/2 cup		
Tally	Score			write in #	write in #

PLEASE TURN OVER



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<i>(office use only)</i>	<i>(office use only)</i>	Food Type	serving size	servings/week	servings/day
	30	Bread, white or whole grain	1 slice		
	120	Waffle or pancake	1 large		
	50	Muffin, biscuit, cornbread	1 medium		
	40	Rolls, buns	½		
	225	Egg McMuffin	1		
	130	Fast food cheeseburger or hamburger	1		
	110	Enchilada or bean burrito	1		
	125	Creamed fish and meats	1 cup		
	130	Shellfish, cooked	4 oz		
	200	Canned salmon with bones	½ cup		
	200	Sardines, smelts, herring	½ cup		
	100	Fudgesicle	1		
	125	Custard pie	1 slice		
	175	Ice cream or ice milk	1 cup		
	190	Pudding with milk	½ cup		
	200	Frozen yogurt	1 cup		

Please list below any dietary supplements (single and multi-vitamins, calcium, herbal etc.) you take daily/weekly, including the brand name, amount (mg) per dose and total number of doses per day (or per week if not taken daily).

1. _____
2. _____
3. _____
4. _____
5. _____



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Calcium/Vitamin D Analysis

Study ID #: _____

Date: ____/____/____

VITAMIN D FFQ/DIET RECALL FORM: Please record how many times you ate/drank this specific food/beverage in the past week and month (4 weeks) and what the typical serving sizes were each time you consumed it? (Please use measuring cup to estimate serving sizes)

Food / Beverage	Frequency/ # of servings in the past week (past 7 days)	Frequency/ # of servings in the past 4 weeks (past 28 days)	Typical serving size(s) each time	Comments
Supplements/multivitamins (Some calcium supps contain vit D)				Type: Brand: Amt. of vit D:
Milk				Type: Don't forget about milk in sauces/casseroles.
Milk beverages (latte, mocha, cappuccino, etc.)				
Soy milk				Brand:
Chocolate milk				
Ice cream				Brand/type:
Whipped cream /Coolwhip				Which one:
Yogurt				Brand:
Cheese (Consider cheese alone & in mixed dishes, such as enchiladas, pizza, casseroles, pasta, etc.)				Note type(s) of cheese:
Butter				
Margarine				
Eggs				Excluding egg whites. Don't forget about omelets, soufflés, frittatas and quiche.
Fish				Don't forget about sushi/sashimi pieces:
Salmon				
Mackerel				
Tuna				
Sardines				
Catfish				
Cod liver oil (NOT including omega 3 supplements)				Note any fish oil sup.
Other				
Mushrooms				Brand:
Liver				
Ready-to-eat cereals				Type/brand:
Ensure or slim fast				Which beverage:
Vit D fortified OJ				
Other vit D fortified food/beverage				

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.

- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT
or GUARDIAN (for participants under the age of majority) _____

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Canadian Society for Exercise Physiology

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continued on other side...

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. *Research Quarterly for Exercise and Sport*, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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.

_____ 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.

_____ 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?

_____ hours per day
_____ minutes 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.

_____ 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?

_____ 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?

_____ 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?

_____ days per week

No walking from place to place



*Skip to PART 3: HOUSEWORK,
HOUSE MAINTENANCE, AND
CARING FOR FAMILY*

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?

_____ 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?

_____ 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?
- ____ 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?
- ____ 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?
- ____ 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?

_____ 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.

Bone-Specific Physical Activity Questionnaire (BPAQ)

Sport/Activity	Sport/Activity	Sport/Activity
Aerobics (High Impact)	Resistance Training (Lower body)	*other-Low Impact
Aerobics (Low Impact)	Rollerblading	*other-Moderate Impact
Australian Rules football	Rowing	*other-High Impact
Badminton	Rugby (football)	
Ballet	Running/jogging	
Baseball	Scuba	
Basketball	Shot Put (throwing events)	
Cheerleading	Skate boarding	
Cricket	Skiing	
Cross-country	Soccer (aka football)	
Cycling	Softball	
Dancing	Squash	
Diving	Stairmaster	
Field Hockey	Surfing	
Flag Football	Swimming	
Golf	T-ball	
Gymnastics	Table Tennis	
Horse-riding	Tennis	
Ice Hockey	Touch football	
Ice-skating (Figure/Dance)	Track	
Judo	Triathlon	
Jump rope	Ultimate	
Kung Fu	Volleyball	
Lacrosse	Walking/hiking	
Lawn Bowls	Waterskiing	
Netball	Windsurfing	
Power lifting	Yoga/Pilates	
Racquet ball		

OU Bone Laboratory



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Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:
-------------	-------

2. Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the last 12 months and indicate the average frequency (sessions per week)?

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

Activity: _____ Frequency (per week): _____

BONE-SPECIFIC PHYSICAL ACTIVITY QUESTIONNAIRE

Developed by B.K. Weeks and B.R. Beck

Griffith University, QLD, Australia



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Subject ID: _____ Date: ____/____/____

	Time Outdoors			Amount of Skin Exposed			
	<5 min	5-30 min	>30min	Hands and face	Hands, face, arms	Hands, face, legs	Bathing suit
Monday	0	1	2	1	2	3	4
Tuesday	0	1	2	1	2	3	4
Wednesday	0	1	2	1	2	3	4
Thursday	0	1	2	1	2	3	4
Friday	0	1	2	1	2	3	4
Saturday	0	1	2	1	2	3	4
Sunday	0	1	2	1	2	3	4

Fig. 1. Scoring for the weekly sun exposure recall questionnaire. Shown in gray are the values ascribed to each category and the method for deriving the Sun Exposure Score (sum of the daily products of Time Outdoors and Skin Exposure). The range of the Sun Exposure Score is from the 0 (lowest amount of time spent outdoors and lowest amount of skin exposed) to a maximum score 56 (outdoors for more than 30 min in a bathing suit every day). (Hanwell et al., 2010)



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Appendix C: Recruitment Material



Female Participants Needed

For the research study, "Racial/Ethnic Differences in Bone Status, Muscle Function, and Fat Mass, in Young and Middle-Aged Premenopausal Women Belonging to Caucasian, South-Asian, and East-Asian Backgrounds"

Michael Bemben, PhD, Principal Investigator

Help us study

- **Fat mass**
- **Muscle mass and strength**
- **Bone mineral density (Bone strength)**

To Participate

- Women 18 - 45 years old
- Potential subjects must have no disabilities preventing them from strength and power testing
- No joint replacement/metal implants
- Weight less than 300 lbs.

3 visits required

Total time commitment about 3 hours and 30 min

Location: Department of Health and Exercise Science, Neuromuscular Lab and Bone Lab, University of Oklahoma

If you are eligible and interested, please contact:

Japneet Kaur, Japneet.Kaur-1@ou.edu or text: 405-420-8540

Department of Health and Exercise Science

The University of Oklahoma is an equal opportunity institution. IRB #9314

Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu	Name: Japneet Kaur Email: japneet.kaur-1@ou.edu
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Mass e-mail script

We are looking for women aged 18-45 years old to participate in a research study titled "Racial/Ethnic Differences in Bone Status, Muscle Function, and Fat Mass, in Young and Middle-Aged Premenopausal Women Belonging to Caucasian, South-Asian, and East-Asian Backgrounds, conducted by Michael Bembem, PhD, Principal Investigator. Potential subjects must have no disabilities preventing them from lower body strength and power testing and hand grip testing, and no joint replacements or metal implants prior to participating in this study.

This study will include 3 visits to the Neuromuscular and Bone Labs at the University of Oklahoma with the first visit lasting approximately 1.5 hour, visit 2 (Goddard Health Center for a blood draw) will last about 20 minutes, and visit 3 lasting approximately 1.5 hour. On the first visit, all participants will complete paperwork consisting of informed consent, a Health Insurance Portability And Accountability Act Privacy Form, physical activity readiness questionnaire, health status questionnaire, menstrual history questionnaire, an ethnicity identification form, calcium and food frequency questionnaires, sun exposure questionnaire, activity questionnaires, and inclusion/exclusion criteria.

The second visit will involve a visit to Goddard Health Center for a single blood draw by a registered nurse or phlebotomist. On the third and final visit, height and weight will be measured using a standard stadiometer and an electronic scale, followed by a urine sample to assess pregnancy status and hydration status. Then, participants will be tested using DXA and pQCT for determination of their total and regional body composition and bone mineral density. Next, upper and lower body muscle strength will be assessed by hand grip test, vertical jumps, and two leg press. Information regarding your results will be provided at the end of the study upon request.

If you are interested in this study or for more information, please contact Japneet Kaur via email: Japneet.Kaur-1@ou.edu or text: 405-420-8540.

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The OUHSC IRB has approved the content of this message but not the method of distribution. The OUHSC IRB has no authority to approve distribution by mass email.



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Facebook Post

RACIAL/ETHNIC DIFFERENCES IN BONE STATUS, MUSCLE FUNCTION, AND FAT MASS, IN YOUNG AND MIDDLE-AGED PREMENOPAUSAL WOMEN BELONGING TO CAUCASIAN, SOUTH-ASIAN, AND EAST-ASIAN BACKGROUNDS

We are currently looking for women aged 18-45 years old to participate in a research study conducted by Michael Bembien, PhD, Principal Investigator. Potential subjects must have no disabilities preventing them from lower body strength and power testing and hand grip testing, and no joint replacements or metal implants prior to participating in this study.

This study requires 3 visits for a total time commitment of about 3.5 hours. Participants will be tested using DXA and pQCT for determination of their total and regional body composition and bone mass. Lower body muscle power will be assessed by 3 vertical jumps. Lower limb strength will be assessed by a two-leg press, and upper limb muscle strength will be assessed using handgrip test. Information regarding your results will be provided at the end of the study upon request.

If you are interested in this study and for more information, please contact Japneet Kaur via email: Japneet.Kaur-1@ou.edu. You may also leave a comment below or send a personal message.

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IRB APPROVAL DATE: 05/09/2018

Telephone script

1. My name is Japneet and I am a graduate student from the University of Oklahoma.
2. Is ___ available?
 - (If yes) I was informed that you may be interested in participating in a study investigating racial/ethnic differences in bone, muscle and fat. Is this correct? (if yes go to question 3).
 - (If no) Is this the correct number to reach ___?
 - (If no) I apologize, please have a great day.
 - (If yes) Do you know a better time for me to call back to reach ___?
 - (if yes/no) Thank you for your time.
 - (If yes) I was calling to give you more information about the study and the requirements of being a subject.
3. Do you have time to talk right now?
 - (If no) What is a better time for me to contact you?
 - (If yes) Let me tell you a little about the study. The purpose of this study is to determine group differences and relationships between bone density and strength, muscle mass and strength, and fat mass, in pre-menopausal women aged 18-45 years belonging to three different racial/ethnic groups: Caucasians, South-Asians, East-Asians. I can give you more exact details if you wish. However, before we proceed, I have to tell you that I will be collecting your answers, if for some reason you chose to not participate, or you do not qualify to participate your name, phone number and the reason you will not be participating will be recorded. This is just for assurance that we are treating all possible subjects fairly, thus your information will be kept private and only used for this research study. If you do not wish to answer questions over the phone I would be more than happy to meet with you in person at the Neuromuscular laboratory, Department of Health and Exercise Science, on the Norman campus, or I can send you more information via e-mail. Please remember that your participation should be voluntary. Would you like to continue?
 - (If no) I thank you for your time.
 - (if yes)
 - First I am going to ask you a series of questions to determine your eligibility.
4. How old are you?
 - (If not in age range) Thank you for your time.
 - (if they are in age range) Let me ask some questions about the inclusion criteria.
5. Are you pregnant or breast-feeding?
6. Do you have any cardiovascular disease or uncontrolled hypertension?
7. Do you have any joint replacements, or any other metal implantation in their hip and spine?
8. Do you have any disabilities or disorders that may prevent you from participating?
9. Do you have any recent surgery, fracture, and open wounds?
10. Are you currently smoking or had smoked regularly within the past 6 months?
 - (if yes) Thank you for your time



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- (if no) Thank you for your response, at this time you (do/do not) qualify for this study.

13. Do you meet the criteria to participate in this study?

- (if yes) Thank you for your time.
- (if no) Thank you for your responses, at this time you (do/do not) qualify for this study.

If they qualify:

Do you have time for me to tell you about what you will be expected to do for this study?

(if no) When is a better time for me to call back? Thank you for your time.

(if yes) proceed to describing the study

This study will include 3 visits to the Neuromuscular and Bone Lab at the University of Oklahoma with the first visit lasting approximately 1.5h, second visit for about 20 minutes, and visit 3 will last approximately 1 hour and 30 minutes. On the first visit, all participants will complete paperwork consisting of informed consent, a health insurance portability and accountability act (HIPAA) form, physical activity readiness questionnaire (PAR-Q), health status questionnaire, menstrual history questionnaire, ethnicity identification form, calcium and food frequency questionnaires, sun exposure questionnaire, activity questionnaires, and inclusion/exclusion criteria. The second visit will involve a visit to Goddard Health Center for a single blood draw by a registered nurse or phlebotomist. On the third and final visit, subject's height and weight will be measured using a standard stadiometer and an electronic scale, followed by a urine sample to assess pregnancy status and hydration status. Then, participants will be tested using DXA and pQCT for determination of their total and regional body composition and bone mineral density. Next, upper and lower body muscle strength will be assessed by hand grip test, vertical jumps, and two leg press. Information regarding your results will be provided at the end of the study upon request.



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Verbal Recruitment Script

Hello, my name is Japneet Kaur, and I am a graduate student in the Department of Health and Exercise Science at the University of Oklahoma. I invite you to participate in a research study entitled "Racial/ethnic differences in bone status, muscle function, and fat mass, in young and middle-aged premenopausal women belonging to Caucasian, South-Asian, and East-Asian backgrounds".

We are looking for women aged 18-45 year old. Potential subjects must have no disabilities preventing them from lower body strength and power testing and hand grip testing, and no joint replacements or metal implants prior to participating in this study. This study will include 3 visits to the Neuromuscular and Bone Labs at the University of Oklahoma with the first visit lasting approximately 1.5 hours, visit 2 (Goddard Health Center for a blood draw) will last about 20 minutes, and visit 3 lasting approximately 1.5 hour. On the first visit, all participants will complete paperwork consisting of informed consent, a health insurance portability and accountability act (HIPAA) form, physical activity readiness questionnaire (PAR-Q), health status questionnaire, menstrual history questionnaire, an ethnicity identification form, calcium and food frequency questionnaires, sun exposure questionnaire, activity questionnaires, and inclusion/exclusion criteria. The second visit will involve a visit to Goddard Health Center for a single blood draw by a registered nurse or phlebotomist. On the third and final visit, subject's height and weight will be measured using a standard stadiometer and an electronic scale, followed by a urine sample to assess pregnancy status and hydration status. Then, participants will be tested using DXA and pQCT for determination of their total and regional body composition and bone mineral density. Next, upper and lower body muscle strength will be assessed by hand grip test, vertical jumps, and two leg press. Information regarding your results will be provided at the end of the study upon request.

There is a slight possibility of mild soreness because of the strength testing. There is also a slight risk of bruising from the blood draw. This research study involves exposure to 4 DXA and 3 pQCT scans, which are types of x-ray procedures, however the amount of additional radiation to which you will be exposed is approximately 1% of the amount of radiation to which we are exposed annually from background sources such as the Earth and Sun. Women must not be and should not become pregnant nor breast-feed an infant while in this study, which may involve risks to an embryo, fetus or infant, including birth defects which are currently unforeseeable.

I would be happy to answer any additional questions that you may have about the study. Thank you!



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Appendix D: Package Inserts for Assays



Immunoenzymetric assay for the *in vitro* quantitative measurement of 25-hydroxyvitamin D₂ and D₃ (25OH-D₂ and 25OH-D₃) in serum.

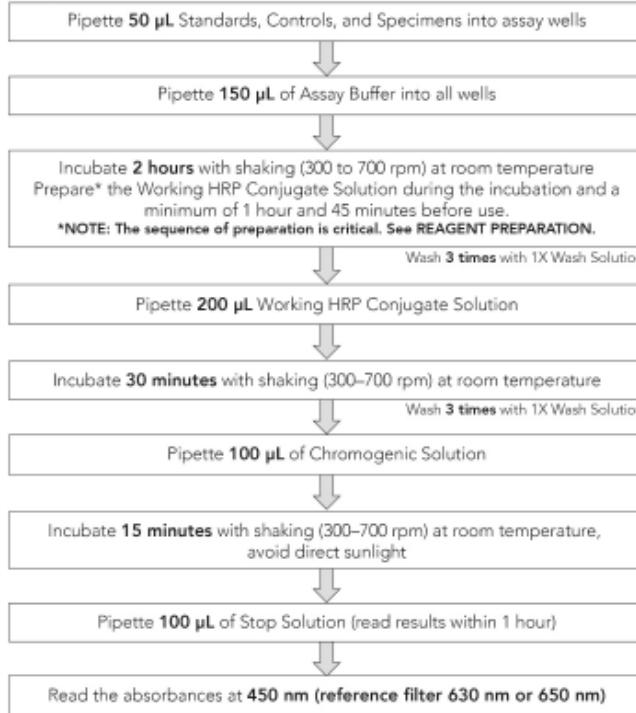
R_x ONLY

SUMMARY

Reagents and Samples Preparation

- Dilute Wash Buffer Concentrate 1:200 with DI Water
- Reconstitute Standards, Controls with deionized or distilled water)

Assay Procedure





INTENDED USE

The MicroVue 25-OH Vitamin D EIA Test is intended for the quantitative determination of 25-hydroxyvitamin D₂ and D₃ (25OH D₂ and 25OH D₃) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.

SUMMARY AND EXPLANATION

Vitamin D is the generic term used to designate Vitamin D₂ or ergocalciferol and Vitamin D₃ or cholecalciferol. Humans naturally produce Vitamin D₃ when the skin is exposed to ultraviolet sun rays. In the liver mainly, Vitamin D₃ is metabolised into 25-Hydroxyvitamin D₃ (25OH D₃) which is the main form of Vitamin D circulating in the body. 25OH D₃ is a precursor for other Vitamin D metabolites and has also a limited activity by itself. The most active derivative is 1, 25-hydroxyvitamin D₃, produced in the kidney (or placenta) by 1-hydroxylation of 25OH D₃. 25OH Vitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralisation. 25OH Vitamin D might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland ...) and endocrine gland (parathyroid glands, beta cells ...).

Vitamin D₃ and Vitamin D₂ are also available by ingestion through food or dietary supplementation. As Vitamin D₂ is metabolised in a similar way to Vitamin D₃, both contribute to the overall Vitamin D status of an individual. It is the reason why it is very important to measure both forms of 25OH Vitamin D equally for a correct diagnosis of Vitamin D deficiency, insufficiency or intoxication.

Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporosis, cancer and pregnancy outcomes. The measurement of both 25OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in patients. Vitamin D intoxication has been shown to cause kidney and tissue damages.

PRINCIPLE OF THE PROCEDURE

The MicroVue 25-OH Vitamin D EIA is a solid phase Enzyme Linked Immunosorbent Assay performed on Microtiter plates. During a first 2-hour incubation step, at room temperature, total 25OH Vitamin D (D₂ and D₃) present in standards, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After 1 washing step, a fixed amount of 25OH Vitamin D-labelled with biotin in presence of horseradish peroxidase (HRP) compete with unlabelled 25OH Vitamin D₂ and 25OH Vitamin D₃ present on the binding sites of the specific monoclonal antibody. After a 30-minute incubation at room temperature, the Microtiterplate is washed to stop the competition reaction. The Chromogenic Solution (TMB) is added and incubated for 15 minutes. The reaction is stopped with the addition of Stop Solution and the Microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH Vitamin D (D₂ and D₃) concentration.

A calibration curve is plotted and the total 25OH Vitamin D (D₂ and D₃) concentrations of the samples are determined by dose interpolation from the calibration curve.

REAGENTS AND MATERIALS PROVIDED

MicroVue 25-OH Vitamin D EIA contains the following:

A 25-OH Vitamin D Standard (Calibrator 0) Lyophilized. Zero standard is biological matrix (human plasma) with gentamycin and ProClin®. Reconstitute with 2 mL DI water.	Part A	1 ea x 2 mL (Std A)
B-F 25-OH Vitamin D Standards B-F (Calibrators 1-5) Lyophilized. Horse serum with gentamycin and Proclin®. Reconstitute each vial with 1 mL DI water.	Part B-F	1 ea x 1 mL (Std B-F)
L 25-OH Vitamin D Control (Control 1) Lyophilized. Human serum with ProClin®. Reconstitute with 1 mL DI water.	Part 4219716	1 ea x 1 mL
H 25-OH Vitamin D Control (Control 2) Lyophilized. Human serum with ProClin®. Reconstitute with 1 mL DI water.	Part 4219717	1 ea x 1 mL
1 Microassay Plate (Microtiterplate) Microtiter plate with 96 Mab anti-25OH Vitamin D ₂ and D ₃ coated wells.	Part 4219708	12 x 8 wells
2 Stop Solution Contains 1M Hydrochloric Acid (HCl).	Part 5504	12 mL
3 200X Wash Buffer Concentrate (Wash Solution) Contains TRIS-HCl. Dilute with DI water.	Part 4219711	10 mL
4 TMB Substrate (Chromogenic Solution TMB) Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB).	Part 5804	12 mL
5 Biotinylated 25-OH Vitamin D (Concentrated Conjugate) 25OH Concentrated Conjugate. Dilute with Reconstitution Solution.	Part of 4119703	0.4 mL
6 Concentrated HRP Contains Concentrated HRP.	Part 4119713	0.2 mL
7 Reconstitution Solution (Conjugate Buffer) Ready to use. Conjugate Buffer with casein and ProClin®.	Part 4119705	30 mL
8 Assay Buffer (Incubation Buffer) Ready to use. Incubation Buffer with casein and ProClin®. ProClin® is a registered trademark of Rohm and Haas Company.	Part 4219713	20 mL

Note: Use 25-OH Vitamin D Standard A (Calibrator 0) for dilution of samples with values above the highest standard.

No international reference material is available

MATERIALS REQUIRED BUT NOT PROVIDED

The following material is required but not provided in the kit:

- Deionized or distilled water
- Pipettes for delivery of: 50 µL, 150 µL, 200 µL and 1 mL (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Plate shaker (300 to 700 rpm)

- Washer for Microassay Plates
- Microassay Plate reader capable of reading at 450 nm and 650 nm or 630 nm (bichromatic reading)

WARNINGS AND PRECAUTIONS

Safety

- *For in vitro* diagnostic use only.
- The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
- All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
- Avoid any skin contact with all reagents. Stop Solution contains HCl. In case of contact, wash thoroughly with water.
- Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2°C to 8°C.
- After reconstitution, standards and controls are stable for eight weeks at 2°C to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 4 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash Solution should be used on the same day.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

REAGENT PREPARATION

Wash Buffer

Prepare an adequate volume of Working Wash solution by adding 199 volumes of DI water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash Solution at the end of the day.

Standard A

Reconstitute the Standard A with 2 mL distilled water.

Standards B-F

Reconstitute the Standards B-F with 1 mL distilled water.

Controls

Reconstitute the Controls with 1 mL distilled water.

Working HRP Conjugate Solution

The Working HRP Conjugate Solution is to be prepared during the 2-hour incubation and at a minimum of 1 hour and 45 minutes before use.

Prepare an adequate volume of Working HRP Conjugate Solution by mixing the three (3) reagents in the following sequence:

1. Reconstitution solution (Conjugate buffer)
2. Biotinylated 25-OH Vitamin D (Concentrated conjugate)
3. Vortex
4. Concentrated HRP
5. Vortex

The order of addition of the three (3) reagents is critical and should be rigorously respected to obtain reproducible Optical Densities.

Prepare an adequate volume of Working HRP Conjugate Solution according to the number of used strips, as indicated below:

- For example, for 6 strips (48 wells): 100 μ L of Concentrated Conjugate and 50 μ L of Concentrated HRP to 10 mL of Conjugate Buffer.
- Use a vortex to homogenize.
- Until its use keep the working HRP conjugate at room temperature and avoid direct sunlight or use a brown glass vial for its preparation.
- The preparation of working HRP conjugate is not stable and must be discarded if not used.

Number of Strips	Volume of Reconstitution Solution (mL)	Volume of Biotinylated 25OH Vitamin D (μ L)	Volume of Concentrated HRP (μ L)
1	3	30	15
2	5	50	25
3	6	60	30
4	8	80	40
5	9	90	45
6	10	100	50
7	12	120	60
8	14	140	70
9	16	160	80
10	18	180	90
11	20	200	100
12	22	220	110

SPECIMEN COLLECTION AND STORAGE

This kit is suitable for serum samples.

Serum samples must be kept at 2°C to 8°C.

If the test is not run within 24 hrs, sampling and storage at -20°C is recommended.

Avoid subsequent freeze-thaw cycles.

ASSAY PROCEDURE

Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Bring all the reagents to room temperature prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform standards, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the TMB Substrate and the Stop Solution, avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
 - To avoid drift, the time between pipetting of the first standard and the last sample must be limited to the time mentioned in section *SPECIFICITY (Time delay)*.
- Prepare a standard curve for each run; do not use data from previous runs.
- Dispense the TMB Substrate within 15 minutes following the washing of the Microassay Plate.
- During incubation with TMB Substrate, avoid direct sunlight on the Microassay Plate.

Procedure

1. Select the required number of Microassay Plate strips for the run. The unused Microassay Plate strips should be resealed in the bag with a desiccant and stored at 2°C to 8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µL of each Standard, Control and Sample into the appropriate wells.
4. Pipette 150 µL of Assay Buffer into all the wells.
5. Incubate for 2 hours at room temperature, on a plate shaker (300 to 700 rpm)
6. Prepare the Working HRP Conjugate Solution once the incubation is started (within 15 minutes).
7. Aspirate the liquid from each well.
8. Wash the plate 3 times by:
 - Dispensing 0.4 mL of Wash Solution into each well
 - Aspirating the content of each well
9. Pipette 200 µL of the Working HRP Conjugate Solution into each well. Incubate the Microassay Plate for 30 minutes at room temperature, on a plate shaker (300 to 700 rpm).
10. Aspirate the liquid from each well.
11. Wash the plate 3 times by:
 - Dispensing 0.4 mL of Wash Solution into each well
 - Aspirating the content of each well
12. Pipette 100 µL of the TMB Substrate into each well within 15 minutes following the washing step.
13. Incubate the Microassay Plate for 15 minutes at room temperature, on a plate shaker (300 to 700 rpm), avoid direct sunlight.
14. Pipette 100 µL of Stop Solution into each well.
15. Read the absorbances at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section Interpretation of Results.

INTERNAL QUALITY CONTROL

- If the results obtained for Control L and/or Control H are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.

- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

INTERPRETATION OF RESULTS

Calculation of Results

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. Calculate for each standard, control and sample:

$$B/B_0(\%) = \frac{\text{OD (Standard B-F, Control or Sample)}}{\text{OD (Standard A (zero calibrator))}} \times 100$$

4. Using either linear-linear or semi-logarithmic graph paper, plot the (B/B₀(%)) values for each standard point as a function of the 25OH Vitamin D concentration of each standard point. Reject obvious outliers.
5. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
6. By interpolation of the sample (B/B₀ (%)) values, determine the 25OH Vitamin D concentrations of the samples from the calibration curve.

TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Standard	Absorbance (OD)	Result (ng/mL)
A	2.66	0
B	2.39	5.3
C	1.83	15
D	1.46	25.7
E	0.81	54.3
F	0.21	133

EXPECTED VALUES

Dietary intake, race, season and age are known to affect the normal levels of 25OH Vitamin D₃. Each laboratory should establish its own range based on their local population. Review of the current literature has suggested the following ranges for the classification of 25OH Vitamin D status:

Level	ng/mL
Deficient	<10
Insufficient	10-29
Sufficient	30-100
Potential Toxicity	>100

REFERENCE RANGE

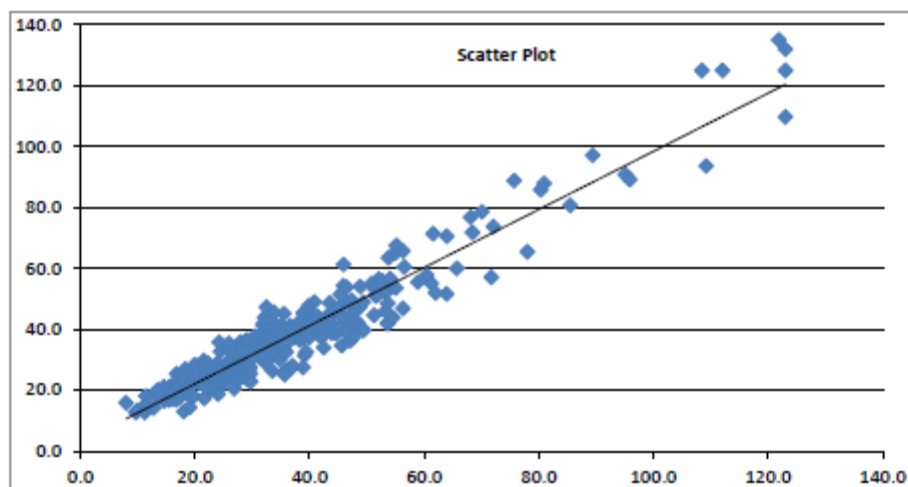
Reference ranges have been established based on 150 apparently healthy individuals. The individual patient serum samples used were obtained from a certified commercial source and were collected from an FDA Licensed Donor Center with informed consent. 50 samples were from Northern U.S. (Pennsylvania), 50 samples were from Central U.S. (Tennessee), and 50 samples were from Southern U.S. (Florida). Samples collected in the winter months (January-March), were between the ages of 21-92 years old and included both light skin and dark skin populations. The samples collected were not taking vitamin D supplements, had no family history of parathyroid, or calcium regulatory disease, had no history of Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery, and were not taking any medications known to affect absorption or catabolism of Vitamin D. The following table is the summary or results:

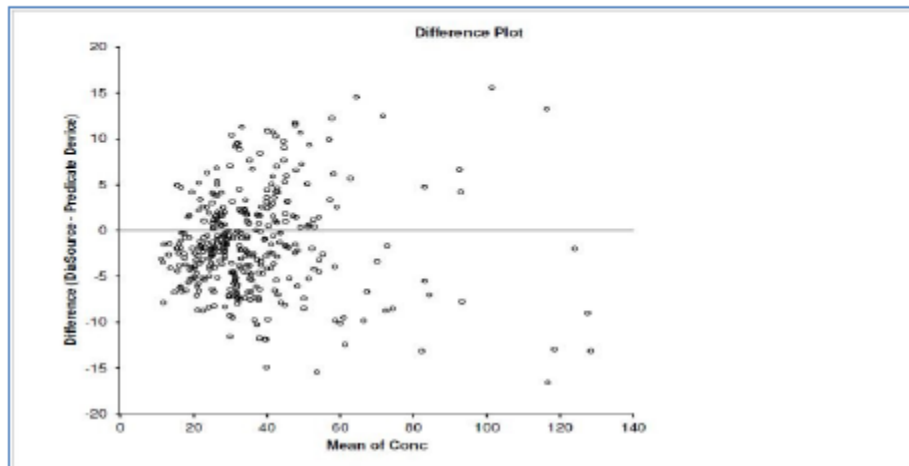
Concentration	Florida	Tennessee	Pennsylvania	Overall
Highest Conc. (ng/mL)	88.6	71.7	54.6	88.6
Lowest Conc. (ng/mL)	6.1	4.9	5.9	4.9
Median Conc. (ng/mL)	20.8	17.2	14.3	17.3

Only Central 95% (2.5% - 97.5%) of the results observed were used.

METHOD COMPARISON

The performance of the MicroVue 25-OH Vitamin D EIA Test was determined by conducting a correlation study tested at three different sites using a total of 356 samples. The samples were tested on both the MicroVue 25-OH Vitamin D EIA Test and a commercially available 25OH Vitamin D EIA test. The results ranged from 8.0ng/mL to 123.0ng/mL, the correlation coefficient between the two methods was 0.917, with the 95% confidence interval of 87.6% to 93.6%, a slope of 0.954 and the y-intercept of 3.05. The following graphs summarize the results:





PERFORMANCE OF THE TEST

Limitation of the Test

1. The test is an aid in the diagnosis and is to be used in conjunction with clinical findings.
2. The performance of this assay has not been established in a pediatric population.
3. Samples suspected of containing concentrations above the highest calibrator should be assayed in dilution.
4. Hemolysed samples should not be used.

Limits of Detection

The Limit of Blank (LOB), Limit of Detection (LOD), and the Limit of Quantitation (LOQ), were determined in accordance with the CLSI guideline EP17-A.

- The LOB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LOB was calculated to be 1.69 ng/mL.
- The LOD was calculated as described in the guideline. The LOD was calculated to be 2.81 ng/mL.
- The LOQ was calculated by testing 5 samples of low value 14 times in different test.
- The LOQ was calculated to be 4.39 ng/mL with CV of 20%.

SPECIFICITY

Cross Reactivity

Cross reactivity of the MicroVue 25-OH Vitamin D EIA was determined by testing sera with spiked and unspiked cross reactants. The results are summarized in the following table:

Compound and Concentration	% Cross Reaction
25 OH-Vitamin D ₃ at 10 ng/mL	100
25 OH-Vitamin D ₂ at 10 ng/mL	86
1, 25(OH) ₂ -Vitamin D ₃ at 200 ng/mL	20
1, 25(OH) ₂ -Vitamin D ₂ at 690 ng/mL	1.9
Vitamin D ₃ at 200 ng/mL	2.9
Vitamin D ₂ at 200 ng/mL	1.3

Compound and Concentration	% Cross Reaction
24,25(OH) ₂ -Vitamin D ₃ at 20 ng/mL	>100
25,26(OH) ₂ -Vitamin D ₃ at 4 ng/mL	>100
3-epi-25OH-Vitamin D ₃ at 20 µg/mL	0.1

Interfering Substances

The effect of potential interfering substances on samples using the MicroVue 25-OH Vitamin D EIA Test was evaluated. Different levels of Hemoglobin, Bilirubin, Triglyceride, Vitamin C, Bilirubin Conjugate and Unconjugated and Zemplar in serum samples were tested on samples with different 25OH Vitamin D concentrations. Our acceptance criteria was to have interference of less than 10%. The tested substances did not affect the performance of the MicroVue 25-OH Vitamin D EIA Test.

Substance	25OH Vitamin D (ng/mL)	Concentration of Interferent (mg/dL)	Mean % Variation
Hemoglobin	7.6	250	-0.6%
		500	
	29.3	250	
		500	
	42.5	250	
		500	
Bilirubin Conjugated	6.0	50	-3.4%
		100	
	21.5	50	
		100	
	38.6	50	
		100	
Bilirubin Unconjugated	7.6	50	2.5%
		100	
	29.3	50	
		100	
	42.5	50	
		100	
Triglyceride	7.6	7.5	-4.3%
		125	
		250	
		500	
	29.3	7.5	
		125	
		250	
		500	
	42.5	7.5	
		125	
		250	
		500	
Vitamin C	6.0	1	2.5%
		10	
		100	

Substance	25OH Vitamin D (ng/mL)	Concentration of Interferent (mg/dL)	Mean % Variation
	21.5	1	
		10	
		100	
	38.6	1	
		10	
		100	
Biotin	8.7	0.2	4.7%
		2	
		4	
	19.8	0.2	
		2	
		4	
	36.1	0.2	
		2	
		4	
Zemplar	17.6	0.0013	-4.4%
		0.0025	
		0.0050	
	33.5	0.0013	
		0.0025	
		0.0050	

Precision

The assay precision was calculated by running samples for a span of at least 20 days on three different lots. The results are summarized in the table below:

Intra-assay				Inter-assay			
Sample	N	<X> ± SD (ng/mL)	C.V. (%)	Sample	N	<X> ± SD (ng/mL)	C.V. (%)
A	24	5.5 ± 0.4	7.8	A	39	17.7 ± 1.3	7.4
B	35	27.4 ± 1.5	5.7	B	10	26.3 ± 1.2	4.7
C	35	43.0 ± 1.2	2.7	C	10	42.1 ± 1.8	4.3
D	24	81.2 ± 2.0	2.5	D	21	85.4 ± 7.8	9.2

SD: Standard Deviation, CV: Coefficient of variation

Reproducibility

The reproducibility of the assay was done by testing three samples in duplicate for five days, twice a day, at three sites with two technicians per site. The mean results are summarized in the table below:

Sample	n	ng/mL		Within-Run	Between-Run	Between-Day	Between-Tech	Between-Site	Total
1	57	25.5	SD	0.22	0.61	0.98	1.54	2.21	2.59
			CV	0.3%	0.9%	3.8%	6.0%	8.7%	10.2%

Sample	n	ng/mL		Within-Run	Between-Run	Between-Day	Between-Tech	Between-Site	Total
2	57	52.9	SD	0.64	1.57	1.11	2.28	4.29	5.19
			CV	0.9%	2.3%	2.1%	4.3%	8.1%	9.8%
3	57	124.9	SD	1.00	1.74	1.84	3.39	4.98	6.25
			CV	1.4%	2.5%	1.5%	2.7%	4.0%	5.0%

Recovery

Recovery was assessed by adding different levels of 25OH Vitamin D to samples. The results are summarized in the table below:

Recovery Test	
Added 25OH-Vit D ₃ (ng/mL)	Recovery (%)
0	100
25	96
50	92
Added 25OH-Vit D ₂ (ng/mL)	Recovery (%)
0	100
25	105
50	95

Linearity

Two samples with concentrations known to be distributed throughout the measurable range were tested at equidistant dilutions to determine the linear range of the assay. A linear regression analysis was performed. The results are summarized in the following table:

Sample 1

Sample Dilution	Theoretical Concentration (ng/mL)	Measured Concentration (ng/mL)	Slope	Y-Intercept	R ²	Recovery (%)
1/1	96.7	96.7	1.00	-0.30	0.99	100
1/2	48.4	47.6				99
1/4	24.2	24.5				101
1/8	12.1	11.1				92
1/16	6.0	6.2				102

Sample 2

Sample Dilution	Theoretical Concentration (ng/mL)	Measured Concentration (ng/mL)	Slope	Y-Intercept	R ²	Recovery (%)
1/1	122.9	122.9	1.01	0.44	0.99	100
1/2	61.5	64.5				105
1/4	30.7	31.5				103
1/8	15.4	15.0				98
1/16	7.7	7.6				99

The linear range of the assay was found to be 7.7 ng/mL to 122.9 ng/mL.

Time Delay

Time delay test between the last standard and sample dispensing results is shown in the following table.

Time Delay			
	0 min (ng/mL)	10 min (ng/mL)	20 min (ng/mL)
Sample 1	27.9	30.5	30.2
Sample 2	49.5	47.5	49.0

Assay results remain accurate even when Assay Buffer is dispensed 10 and 20 minutes after the standard has been added in the coated wells.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support, contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF

8046 – MicroVue 25-OH Vitamin D EIA Kit



Instructions for Use

FSH ELISA



EIA-1288
96 Wells

IVD

Control 2
range 14.0 - 36.9 mIU/ml



Legal Manufacturer:

DRG

DRG Instruments GmbH, Germany
Division of DRG International, Inc
Frauenbergstr. 18, D-35039 Marburg
Telefon: +49 (0)6421-17000 Fax: +49-(0)6421-1700 50
Internet: www.drg-diagnostics.de
E-mail: drg@drg-diagnostics.de

Distributed by:



10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
18. Some reagents contain Proclin, BND and MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells
Wells coated with anti-FSH monoclonal antibody
2. **Standard (Standard 0-5)**, 6 vials (lyophilized), 1 mL
Concentration: 0; 5; 10; 20; 50; 100 mIU/mL.
Conversion: 6 mIU/mL = 1 ng/mL
The standards are calibrated against 1. International Standard for Follicle Stimulation Hormone (FSH), human recombinant for immunoassay NIBSC code 92/510 see „Preparation of Reagents“
Contain non-mercury preservative.
3. **Enzyme Conjugate**, 1 vial, 11 mL, ready to use
Anti-FSH antibody conjugated to horseradish peroxidase;
Contain non-mercury preservative.
4. **Substrate Solution**, 1 vial, 14 mL, ready to use
Tetramethylbenzidine (TMB).
5. **Stop Solution**, 1 vial, 14 mL, ready to use
contains 0.5M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.

Note: Additional Standard 0 for sample dilution is available on request.

4.2 Material required but not provided

- A microtiter plate calibrated reader (480 ±10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Linear graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

4.4 Reagents Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 1 mL deionized water.

Note: The reconstituted standards are stable for 2 months at 2 °C - 8 °C.
For longer storage freeze at -20 °C.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Only serum should be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection**Serum:**

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C - 8 °C prior to assaying.

Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Standard 0 (mix thoroughly).

6 ASSAY PROCEDURE**6.1 General Remarks**

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **25 μ L** of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense **100 μ L** Enzyme Conjugate into each well. *dest*
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **30 minutes** at room temperature.
6. *dest* **BRIEFLY** shake out the contents of the wells.
7. **RINSE** the wells **5 times** with aqua dest (400 μ L per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure! *BLOT*

8. Add **100 μ L** of Substrate Solution to each well.
9. Incubate for **10 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **50 μ L** of Stop Solution to each well.
11. Determine the absorbance (OD) of each well at **450 \pm 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 mIU/mL)	0.07
Standard 1 (5 mIU/mL)	0.16
Standard 2 (10 mIU/mL)	0.26
Standard 3 (20 mIU/mL)	0.44
Standard 4 (50 mIU/mL)	0.92
Standard 5 (100 mIU/mL)	1.71

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG FSH ELISA the following values are observed:

Population	5% - 95% Percentile [mIU/mL]
Males	0.89 - 11.72
Female	
Follicular Phase	2.0 - 10.0
Mid-cycle	7.0 - 20.0
Luteal Phase	2.0 - 10.0
Post-Menopausal	20.0 - 100.0

Appendix E: Raw Data

ID	ETHNICITY	HEIGHT_cm	WEIGHT_kg	BMI	HGS_R	HGS_L	hgs_avg	JT_POWER	RE_JT_PWR	JT_VEL	JT_HEIGHT	JT_TIME	@1RM_KG	AGE_yr	AGE_GROUP	BMD_TB	BMD_TB_z	prtfat_R_arm	prtfat_L_arm	prtfat_R_leg	prtfat_L_leg	prtfat_total	gfat_R_arm	gfat_L_arm	gfat_R_leg	gfat_L_leg	AG	total_FAT_KG
JK30	1	159.50	48.10	18.91	23.67	23.47	23.57	731.67	15.21	1.33	12.63	0.45	181.82	18.00	1.00	0.975		23.90	25.30	31.40	31.80	24.70	634.00	642.00	2684.00	2603.00	0.20	11.95
JK04	1	160.50	59.50	23.10	19.50	16.80	18.15	776.33	13.05	1.32	11.97	0.50	145.45	19.00	1.00	0.898		38.90	40.50	40.90	40.90	39.20	1306.00	1333.00	4247.00	4139.00	0.41	23.22
JK38	1	162.50	50.20	19.01	31.27	26.87	29.07	653.33	13.01	1.31	14.07	0.54	104.36	19.00	1.00	1.087		21.00	22.40	24.70	25.10	22.00	630.00	599.00	2229.00	2135.00	0.24	11.07
JK12	1	152.00	48.40	20.95	21.93	19.30	20.62	572.00	11.82	1.17	11.90	0.49	81.82	19.70	1.00	0.933		31.00	29.40	40.40	38.10	32.50	875.00	772.00	3298.00	3030.00	0.24	15.63
JK26	1	168.00	76.30	27.03	26.33	19.53	22.93	627.20	8.22	1.23	10.10	0.46	154.55	19.80	1.00	1.012		43.60	45.00	43.10	42.10	39.20	1866.00	1925.00	6255.00	6033.00	0.33	29.88
JK13	1	174.00	64.30	21.24	21.53	19.83	20.68	492.67	7.66	0.77	16.33	0.58	145.45	20.80	1.00	1.260	1.800	34.80	36.20	32.20	32.80	30.50	1360.00	1325.00	3736.00	3717.00	0.30	19.26
JK22	1	162.00	49.10	18.71	24.20	23.07	23.63	614.00	12.51	1.25	9.97	0.45	118.18	20.80	1.00	1.047	0.400	30.50	29.40	30.70	30.40	28.00	844.00	744.00	2545.00	2511.00	0.32	13.77
JK10	1	159.50	52.80	20.75	27.83	23.63	25.73	709.67	13.44	1.37	14.43	0.55	131.82	21.60	1.00	1.113	0.900	29.50	28.80	34.00	33.40	28.70	904.00	844.00	3208.00	3185.00	0.24	15.19
JK16	1	165.50	81.90	29.90	28.13	23.27	25.70	1125.67	13.74	1.40	16.20	0.57	186.36	21.80	1.00	1.274	1.200	37.80	38.20	31.20	30.50	39.30	1764.00	1618.00	3860.00	3871.00	0.74	32.10
JK15	1	157.00	55.90	22.68	23.43	23.03	23.23	593.00	10.61	1.08	13.80	0.53	150.00	21.90	1.00	1.100	0.600	22.50	24.00	27.90	28.40	23.60	693.00	765.00	2847.00	2996.00	0.18	13.14
JK09	1	160.50	59.30	23.02	22.17	24.47	23.32	607.33	10.24	1.03	12.50	0.51	237.27	22.90	1.00	1.149	0.900	30.00	30.20	36.20	36.20	30.90	1125.00	1071.00	3704.00	3721.00	0.26	18.35
JK19	1	169.00	70.30	24.60	21.03	20.37	20.70	589.00	8.38	1.23	14.83	0.55	86.36	24.10	1.00	1.243	1.400	34.60	33.60	35.80	37.80	33.80	1332.00	1305.00	4768.00	4866.00	0.29	23.79
JK07	1	175.00	61.60	20.11	30.20	29.50	29.85	884.33	14.36	1.37	19.07	0.62	218.18	25.50	1.00	1.183	1.200	19.60	20.60	25.60	26.70	21.40	767.00	753.00	3238.00	3258.00	0.16	13.26
JK24	1	162.50	53.50	20.26	25.73	22.53	24.13	612.67	11.45	1.18	10.43	0.46	86.36	25.70	1.00	1.040	0.100	35.20	34.70	36.80	37.90	34.10	1091.00	1099.00	3443.00	3437.00	0.35	18.28
JK49	1	171.00	71.10	24.32	32.40	28.93	30.67	1080.33	14.15	1.33	12.57	0.51	131.82	26.00	1.00	1.236	1.300	33.00	37.10	36.80	36.10	33.00	1243.00	1404.00	4473.00	4537.00	0.28	23.33
JK42	1	163.50	102.40	38.31	30.20	29.23	29.72	1380.33	13.48	1.37	10.07	0.45	159.09	26.10	1.00	1.268	0.300	46.30	47.50	46.00	45.10	47.20	2764.00	2877.00	7703.00	7676.00	0.57	48.02
JK08	1	173.00	64.40	21.52	29.27	28.47	28.87	892.00	13.85	1.40	17.87	0.60	168.18	26.40	1.00	1.181	1.000	21.20	22.30	25.60	23.00	21.50	806.00	796.00	2836.00	2627.00	0.23	13.88
JK01	1	175.00	84.90	27.72	36.00	35.37	35.68	1149.67	13.54	1.38	20.00	0.64	193.41	26.60	1.00	1.280	1.100	26.70	26.60	34.70	32.80	29.90	1301.00	1234.00	5748.00	5397.00	0.27	25.25
JK46	1	171.50	70.00	23.80	19.93	18.47	19.20	800.67	11.44	1.17	10.67	0.47	140.91	27.30	1.00	1.236	1.300	37.80	39.20	40.10	40.10	34.50	1443.00	1564.00	4879.00	4718.00	0.35	24.13
JK03	1	166.00	68.20	24.75	25.43	24.77	25.10	861.33	12.62	1.27	16.37	0.58	239.64	27.60	1.00	1.255	1.600	31.80	34.30	37.90	36.50	32.70	1264.00	1317.00	4883.00	4700.00	0.30	22.31
JK47	1	157.00	48.40	19.64	24.43	24.87	24.65	579.33	11.97	1.21	13.30	0.52	131.82	28.30	1.00	1.100	0.900	24.50	26.30	35.00	33.30	36.20	660.00	675.00	3120.00	3001.00	0.18	12.84
JK60	1	172.00	113.40	38.33	28.97	28.40	28.68	1266.67	11.17	1.13	7.60	0.39	140.91	29.40	1.00	1.358	1.200	46.70	46.70	40.80	40.60	44.50	2668.00	2668.00	8249.00	7737.00	0.75	49.79
JK39	1	161.20	59.30	22.82	22.13	19.80	20.97	651.00	10.98	1.11	8.30	0.41	72.73	29.70	1.00	1.039	-0.200	37.70	38.50	44.50	43.30	38.10	1131.00	1128.00	5146.00	4979.00	0.26	22.56
JK67	1	160.00	54.10	21.13	28.40	24.13	26.27	704.67	13.03	1.31	10.90	0.47	122.73	29.80	1.00	1.181	1.500	33.00	31.70	35.90	36.40	30.10	996.00	955.00	3773.00	3871.00	0.23	16.27
JK65	1	169.00	66.50	23.28	27.80	28.47	28.13	862.67	12.97	1.31	16.23	0.58	150.00	30.90	2.00	1.195	1.100	38.20	28.20	32.50	33.10	27.50	1166.00	1134.00	3952.00	3871.00	0.22	18.29
JK61	1	169.70	76.60	26.60	22.17	24.17	23.17	790.00	10.31	1.05	10.97	0.47	109.09	31.10	2.00	1.304	1.700	43.50	44.10	47.90	48.60	41.60	1985.00	1906.00	7664.00	7601.00	0.29	31.80
JK02	1	169.50	83.60	29.10	32.87	32.67	32.77	1087.00	13.00	1.32	14.80	0.55	231.82	31.30	2.00	1.381	2.200	36.50	39.10	41.50	39.70	39.30	1729.00	1678.00	6353.00	5917.00	0.37	32.93
JK63	1	169.50	75.00	26.10	30.43	28.07	29.25	970.67	12.94	1.32	12.37	0.50	177.27	31.40	2.00	1.343	2.200	33.40	35.70	34.50	36.10	32.40	1457.00	1506.00	4639.00	4838.00	0.37	24.28
JK37	1	172.50	52.00	17.48	25.70	23.73	24.72	625.00	12.02	1.23	12.80	0.51	90.91	31.80	2.00	1.166	1.400	28.10	25.50	32.50	32.30	24.60	800.00	742.00	3045.00	2940.00	0.15	12.83
JK54	1	167.00	73.20	26.25	31.20	26.90	29.05	870.33	11.89	1.20	13.30	0.53	154.55	31.90	2.00	1.179	0.600	28.10	27.90	30.40	31.30	32.10	1249.00	1200.00	3713.00	3725.00	0.51	23.23
JK64	1	164.70	63.40	23.37	30.57	27.87	29.22	828.33	13.07	1.32	12.63	0.51	140.91	32.00	2.00	1.338	2.600	36.60	36.90	37.20	37.50	34.00	1184.00	1165.00	4381.00	4374.00	0.35	21.58
JK70	1	161.50	56.80	21.78	21.53	14.07	17.80	693.00	12.20	1.24	9.70	0.45	118.18	32.60	2.00	1.145	1.000	51.20	30.60	38.70	39.30	31.00	1009.00	934.00	4079.00	4058.00	0.21	17.46
JK77	1	169.50	86.40	30.07	26.27	19.57	22.92	1211.00	14.02	1.42	10.40	0.45	163.64	34.20	2.00	1.479	3.000	35.60	38.50	41.20	38.30	35.30	1823.00	1850.00	6626.00	6110.00	0.35	30.56
JK55	1	178.00	95.10	30.02	29.67	26.47	28.07	1374.00	14.45	1.46	9.27	0.44	150.00	34.50	2.00	1.255	0.400	48.00	45.80	46.40	47.50	47.60	2197.00	2170.00	7407.00	7429.00	0.55	45.05
JK71	1	157.40	74.00	29.87	25.27	22.50	23.88	977.00	13.20	1.35	10.70	0.47	222.73	35.80	2.00	1.309	1.900	41.00	39.10	41.10	41.40	39.20	1618.00	1516.00	5183.00	5362.00	0.42	29.00
JK43	1	162.60	79.10	29.92	30.40	24.43	27.42	1077.00	13.62	1.37	12.27	0.50	150.00	35.90	2.00	1.261	1.200	37.90	38.90	34.10	32.60	36.60	1817.00	1818.00	4572.00	4246.00	0.48	28.95
JK75	1	157.00	64.30	26.09	27.00	24.40	25.70	764.67	11.89	1.20	7.70	0.40	90.91	36.40	2.00	1.223	1.400	36.10	39.30	37.60	38.90	35.50	1354.00	1407.00	4152.00	4047.00	0.36	22.78
JK40	1	157.00	43.50	17.65	22.20	20.63	21.42	514.33	11.82	1.19	14.10	0.54	86.36	37.80	2.00	1.024	0.400	20.20	22.20	30.80	29.30	21.00	493.00	531.00	2343.00	2288.00	0.18	9.19
JK45	1	171.00	92.50	31.63	36.37	34.73	35.55	1179.33	12.75	1.29	12.27	0.51	145.45	38.40	2.00	1.326	1.200	42.00	42.80	43.30	43.30	44.90	2059.00	2095.00	6904.00	6626.00	0.57	41.39
JK73	1	171.00	61.90	21.17	29.93	26.30	28.12	761.67	12.30	1.25	13.60	0.52	127.27	38.60	2.00	1.244	1.800	25.10	25.70	27.00	26.90	24.70	878.00	854.00	2999.00	2926.00	0.35	15.30
JK58	1	176.50	99.50	31.94	26.23	19.93	23.08	1117.67	11.23	1.14	10.60	0.47	139.02	38.90	2.00	1.200	-0.300	48.70	48.70	47.40	47.50	47.20	2655.00					

JK115	2	158.00	46.40	18.59	29.57	21.13	25.35	598.00	12.89	1.34	11.90	0.50	100.00	18.90	1.00	0.934	29.60	32.90	35.90	35.80	30.20	785.00	792.00	3005.00	2873.00	0.24	13.98	
JK25	2	155.50	52.50	21.71	23.07	24.47	23.77	633.67	12.07	1.22	10.50	0.46	86.36	19.00	1.00	0.981	34.30	32.70	34.80	34.10	29.60	969.00	912.00	3108.00	3084.00	0.22	15.56	
JK116	2	154.50	84.40	35.36	14.80	12.27	13.53	922.33	10.93	1.11	8.60	0.42	95.45	19.40	1.00	0.947	46.20	46.20	46.20	44.50	46.60	2151.00	2151.00	6540.00	6173.00	0.54	39.12	
JK76	2	161.00	51.10	19.71	18.67	18.70	18.68	626.67	12.26	1.25	10.80	0.47	118.18	20.00	1.00	1.129	32.10	33.70	34.00	32.90	31.20	843.00	926.00	2808.00	2737.00	0.35	15.96	
JK106	2	159.00	51.90	20.53	25.60	19.87	22.73	611.67	11.79	1.20	10.87	0.47	90.91	20.00	1.00	1.094	32.80	33.60	33.60	34.20	29.00	853.00	817.00	2955.00	2976.00	0.20	15.05	
JK108	2	164.00	52.50	19.52	18.73	21.90	20.32	692.33	13.19	1.33	12.70	0.51	113.64	20.10	1.00	1.156	31.10	26.20	29.50	29.60	27.20	904.00	716.00	2781.00	2443.00	0.26	14.30	
JK110	2	155.00	61.40	21.39	22.03	19.33	20.68	627.00	12.20	1.21	14.20	0.54	131.82	20.30	1.00	1.185	23.50	22.50	26.50	24.90	21.80	630.00	576.00	2399.00	2056.00	0.19	11.16	
JK59	2	155.00	53.00	26.22	24.83	20.73	22.78	856.33	13.59	1.39	8.63	0.42	95.45	20.80	1.00	1.148	48.00	47.70	39.10	37.70	42.80	1719.00	1695.00	3736.00	3707.00	0.62	26.89	
JK32	2	153.50	50.70	21.52	24.37	15.83	20.10	730.00	14.40	1.46	18.87	0.62	131.82	21.00	1.00	1.106	31.40	32.30	26.80	26.00	31.50	850.00	822.00	2235.00	2120.00	0.64	15.98	
JK29	2	158.50	49.80	19.82	23.63	24.57	24.10	617.33	12.40	1.26	14.57	0.54	118.18	21.20	1.00	1.179	24.60	28.40	25.20	24.00	23.60	685.00	746.00	2010.00	1927.00	0.30	11.73	
JK62	2	160.50	54.80	21.27	24.23	19.23	21.73	776.33	14.17	1.44	11.87	0.50	122.73	21.40	1.00	1.167	28.30	29.40	30.20	30.50	27.30	761.00	750.00	3097.00	2971.00	0.25	14.86	
JK107	2	147.50	46.40	21.33	16.87	15.93	16.40	576.33	12.42	1.31	11.60	0.49	159.09	21.60	1.00	1.099	36.50	38.80	35.70	35.70	34.80	870.00	932.00	2636.00	2590.00	0.37	16.09	
JK41	2	171.50	96.40	32.78	24.57	23.70	24.13	1401.67	14.54	1.47	9.87	0.45	154.55	21.70	1.00	1.226	42.70	44.90	36.60	35.60	42.90	2068.00	2214.00	5557.00	5356.00	0.65	41.20	
JK102	2	155.00	51.70	21.52	21.30	17.17	19.23	600.00	11.61	1.20	11.00	0.48	104.55	21.80	1.00	0.996	41.20	41.90	37.50	37.70	36.90	1109.00	1098.00	3354.00	3271.00	0.40	19.08	
JK114	2	158.00	54.60	21.87	27.00	26.53	26.77	709.33	12.99	1.34	14.37	0.54	186.36	22.30	1.00	1.026	28.30	28.30	29.00	29.80	27.30	841.00	867.00	2701.00	2742.00	0.27	14.86	
JK05	2	162.00	63.70	24.27	33.73	30.23	31.98	778.00	12.21	1.24	17.33	0.60	236.59	22.80	1.00	1.275	27.20	26.40	25.80	26.10	28.10	1094.00	977.00	2809.00	2797.00	0.44	17.93	
JK109	2	156.00	63.00	25.89	20.67	19.27	19.97	687.33	10.91	1.11	7.73	0.39	118.18	25.60	1.00	1.273	34.40	39.00	37.00	37.70	32.50	1052.00	1152.00	4121.00	4142.00	0.24	20.51	
JK87	2	170.50	53.20	18.30	16.57	16.20	16.38	617.67	11.61	1.17	9.33	0.43	72.73	25.80	1.00	1.101	35.80	34.10	34.00	35.90	30.90	866.00	817.00	3393.00	3600.00	0.21	16.40	
JK97	2	157.50	46.50	18.75	19.23	15.60	17.42	565.00	12.15	1.23	12.13	0.50	113.64	26.40	1.00	1.137	37.00	35.10	36.20	34.60	31.50	852.00	781.00	2831.00	2558.00	0.30	14.59	
JK101	2	158.00	52.70	21.11	27.10	26.93	27.02	672.00	12.75	1.30	13.70	0.53	181.82	26.00	1.00	1.136	29.80	27.30	30.90	31.30	25.80	732.00	731.00	2949.00	2844.00	0.24	13.68	
JK28	2	168.60	62.70	22.10	24.27	26.03	25.15	737.00	11.75	1.19	12.20	0.52	81.82	27.30	1.00	1.116	43.50	42.90	35.60	34.70	37.40	1540.00	1447.00	3530.00	3416.00	0.48	23.41	
JK52	2	155.50	55.70	23.04	24.17	24.73	24.45	786.33	14.12	1.43	12.80	0.51	109.09	28.00	1.00	1.200	37.60	37.60	37.20	38.50	44.80	1086.00	1076.00	3641.00	3788.00	0.42	21.64	
JK112	2	162.50	68.30	25.87	26.60	25.30	25.95	723.67	10.60	1.15	10.83	0.47	172.73	30.70	2.00	1.158	33.30	31.80	32.90	34.70	35.20	1150.00	1141.00	4014.00	3514.00	0.41	23.93	
JK53	2	156.00	68.10	27.98	17.60	18.23	17.92	924.67	13.58	1.39	13.03	0.52	100.00	33.00	2.00	1.214	37.20	39.20	35.70	35.70	35.80	1531.00	1574.00	3964.00	3760.00	0.45	24.34	
JK113	2	155.00	45.50	18.94	21.00	21.40	21.20	519.00	11.41	1.15	8.37	0.41	100.00	36.10	2.00	1.077	35.40	36.50	38.70	39.00	34.10	804.00	768.00	2853.00	2874.00	0.29	15.43	
JK103	2	159.00	69.00	27.29	32.77	32.23	32.50	933.33	13.53	1.38	16.23	0.58	186.36	37.30	2.00	1.329	31.70	34.10	26.70	24.80	30.00	1318.00	1444.00	2974.00	2747.00	0.60	20.71	
JK95	2	164.00	66.70	24.80	25.93	24.80	25.37	690.33	10.35	1.07	8.73	0.42	90.91	45.70	2.00	1.120	35.60	34.10	37.00	36.90	36.60	1156.00	1030.00	4203.00	4058.00	0.41	24.37	
JK117	2	162.50	56.40	21.36	27.20	21.10	24.15	659.33	11.69	1.18	11.27	0.48	104.33	30.30	2.00	1.087	28.30	28.40	37.50	37.60	32.00	670.00	643.00	3931.00	3956.00	0.27	17.89	
JK118	2	162.00	51.40	19.59	25.60	25.00	25.30	638.70	12.43	1.25	14.83	0.55	90.72	31.50	2.00	0.988	36.70	37.40	33.70	35.30	32.10	818.00	814.00	2962.00	2955.00	0.33	16.50	
JK119	2	162.50	52.60	19.92	27.10	24.70	25.90	775.00	14.73	1.50	12.77	0.51	99.79	31.90	2.00	1.175	36.80	36.90	32.60	32.00	32.40	1077.00	1016.00	2911.00	2819.00	0.42	17.07	
JK120	2	164.50	52.70	19.48	31.10	31.70	31.40	630.30	11.96	1.21	14.07	0.54	72.57	33.30	2.00	1.116	27.40	26.40	30.60	30.00	27.30	735.00	695.00	2683.00	2570.00	0.39	14.40	
JK121	2	165.50	54.50	19.90	22.40	19.70	21.05	625.70	11.48	1.14	12.30	0.50	68.04	34.80	2.00	0.985	38.80	36.50	34.10	32.90	34.50	924.00	891.00	3200.00	3021.00	0.41	18.85	
JK20	3	157.00	81.10	32.90	24.17	21.40	22.78	1164.67	14.36	1.47	11.47	0.48	154.55	18.00	1.00	1.040	44.80	44.30	47.30	46.20	44.90	2237.00	2052.00	6707.00	6476.00	0.51	36.36	
JK21	3	155.00	52.00	21.64	18.27	18.87	18.57	659.33	12.68	1.49	14.23	0.54	127.27	18.80	1.00	0.966	32.40	35.30	40.30	40.50	35.20	923.00	1006.00	3932.00	3857.00	0.28	18.28	
JK51	3	166.00	72.70	26.38	25.83	23.00	24.42	887.33	12.21	1.24	9.37	0.44	95.45	20.10	1.00	1.161	43.40	44.40	41.50	41.20	41.50	1898.00	1839.00	5386.00	5399.00	0.35	30.07	
JK88	3	155.00	80.80	33.63	24.30	22.83	23.57	1046.00	12.95	1.33	9.30	0.44	136.36	20.20	1.00	1.260	46.30	46.40	43.60	44.10	45.20	2176.00	2135.00	6558.00	6574.00	0.46	36.44	
JK44	3	165.50	66.10	23.84	22.40	17.77	20.08	704.67	10.66	1.07	8.00	0.41	104.55	20.80	1.00	1.101	0.200	32.20	35.70	43.60	44.60	36.30	1072.00	1152.00	3750.00	3619.00	0.23	23.98
JK35	3	159.80	61.00	23.89	26.87	26.03	26.45	773.00	12.67	1.29	11.67	0.49	136.36	21.70	1.00	1.108	36.10	35.00	40.10	42.10	37.40	1275.00	1206.00	4524.00	4765.00	0.33	22.75	
JK79	3	157.00	44.00	17.85	22.73	21.73	22.23	557.67	12.67	1.29	16.00	0.55	140.91	21.70	1.00	1.070	29.50	28.60	31.40	31.00	31.60	749.00	659.00	2304.00	2208.00	0.43	13.74	
JK89	3	163.00	43.20	16.26	16.83	18.87	17.85	477.33	11.05	1.13	8.93	0.43	68.18	21.90	1.00	1.016	33.70	33.70	41.60	40.40	32.00	801.00	784.00	3217.00	3216.00	0.22	13.71	
JK91	3	156.00	55.70	22.89	16.37	14.00	15.18	637.00	11.44	1.16	9.17	0.43	90.91	23.50	1.00	1.152	43.70	41.70	42.10	42.30	40.70	1356.00	1248.00	4192.00	4163.00	0.39	22.64	
JK80	3	183.00	75.90	22.66	32.67	28.70	30.68	1018.00	13.41	1.37	12.93	0.52	177.27	24.40	1.00	1.122	33.40	33.60	35.60	35.70	32.60	1481.00	1450.00	4876.00	4837.00	0.34	24.73	
JK83	3	160.60	69.60	26.98	21.30	18.27	19.78	736.67	10.58	1.07	10.03	0.45	150.00	25.10	1.00	1.190	44.80	48.50	43.60	43.50	40.90	1937.00	1951.00	6145.00	6067.00	0.27	28.40	
JK11	3	154.50	54.60	22.87	26.																							

ID	glean_R_arm	glean_L_arm	glean_R_leg	glean_L_leg	ASM_KG	total_LEAN	bmc_total	VAT_vol_cm3	VAT_mass_g	BMD_LS	BMD_LS_z	BMC_LS	BMD_FN_left	BMD_FN_rt	BMD_FN_l	BMD_FN_r	BMC_FN	BMC_FN	TROC_BN	TROC_F	TROC_BMDlt_z	TROC_BMDrt_z	TROC_BMC_l	TROC_BMC_r
JK30	1878.00	1769.00	5490.000	5254.000	14.391	34.273	2083.400	60.000	57.000	1.151		58.470	1.033	1.046			4.180	4.420	0.795	0.859			7.280	9.000
JK04	1939.00	1844.00	5783.000	5647.000	15.213	34.129	1879.200	466.000	440.000	1.001		52.820	0.954	0.992			4.230	4.420	0.673	0.686			6.720	7.520
JK38	2218.00	1931.00	6358.000	5952.000	16.459	36.723	2444.800	57.000	54.000	1.294		73.640	1.032	1.056			4.800	4.890	0.894	0.890			9.360	9.050
JK12	1818.00	1740.00	4539.000	4609.000	12.706	30.513	1958.100	65.000	61.000	1.259		60.170	0.940	0.964			4.040	4.120	0.764	0.815			6.150	7.340
JK26	2285.00	2220.00	7828.000	7871.000	20.204	22.191	1122.200	478.000	451.000	1.054		50.890	0.886	0.871			4.580	4.110	0.771	0.745			8.870	9.400
JK13	2364.00	2166.00	7329.000	7097.000	18.956	42.123	2555.000	142.000	134.000	1.261	0.700	68.250	1.110	1.139	0.400	0.500	5.510	5.580	0.930	0.894	0.600	0.300	11.280	10.770
JK22	1801.00	1671.00	5409.000	5421.000	14.302	33.429	1976.000	13.000	21.000	1.143	0.200	53.530	0.918	0.907	-0.600	-0.700	4.360	4.100	0.705	0.733	-0.900	-0.700	5.530	5.900
JK10	2022.00	1954.00	5865.000	5999.000	15.840	35.671	1976.000	100.000	94.000	1.073	-0.500	49.810	1.054	1.008	0.300	0.000	4.400	4.170	0.806	0.799	-0.100	-0.200	7.620	7.960
JK16	2750.00	2476.00	8103.000	8365.000	21.694	47.093	2548.000	935.000	882.000	1.362	1.000	82.840	1.205	1.179	0.700	0.600	5.240	5.250	1.033	1.032	1.100	1.100	12.710	12.700
JK15	2259.00	2294.00	6975.000	7173.000	18.701	40.639	2003.000	0.000	0.000	1.207	0.500	61.120	1.048	0.989	0.200	-0.200	4.790	4.530	0.895	0.860	0.600	0.300	9.100	8.610
JK09	2461.00	2323.00	6118.000	6128.000	17.030	38.749	2262.000	163.000	154.000	1.311	1.300	70.300	1.068	1.098	0.300	0.500	4.960	5.180	0.798	0.785	-0.300	-0.500	8.710	8.330
JK19	2339.00	2406.00	8054.000	7537.000	20.336	43.892	2704.000	1.000	1.000	1.358	1.300	78.140	1.218	1.177	1.200	0.900	5.870	5.970	0.846	0.868	-0.200	0.000	10.660	11.080
JK07	2962.00	2743.00	8905.000	8471.000	23.081	45.990	2621.000	0.000	0.000	1.266	0.800	78.480	0.993	1.029	-0.200	0.000	4.710	4.870	0.784	0.816	-0.500	-0.200	9.650	11.060
JK24	1872.00	1942.00	5582.000	5306.000	14.702	33.373	1906.000	148.000	139.000	1.225	0.800	58.230	1.015	0.989	0.100	-0.100	4.240	4.000	0.727	0.725	-0.800	-0.800	8.180	7.270
JK49	2364.00	2219.00	7257.000	7583.000	19.423	44.788	2613.000	279.000	264.000	1.291	0.700	66.690	1.096	1.101	0.300	0.400	5.430	5.200	0.812	0.808	-0.500	-0.500	10.570	10.690
JK42	3050.00	3019.00	8623.000	8928.000	23.620	51.269	2495.000	1349.000	1273.000	1.188	-1.100	66.870	1.157	1.186	0.100	0.300	5.310	5.370	0.913	0.903	-0.400	-0.400	9.700	10.410
JK08	2821.00	2604.00	7794.000	8338.000	21.557	48.115	2540.000	40.000	38.000	1.209	0.300	65.830	1.002	0.931	-0.200	-0.700	4.810	4.780	0.796	0.783	-0.400	-0.500	9.290	8.260
JK01	3393.00	3242.00	10319.000	10543.000	27.497	56.271	2809.000	86.000	81.000	1.268	0.100	68.930	1.077	1.038	-0.100	-0.400	5.320	5.040	0.883	0.841	-0.200	-0.600	12.960	12.210
JK46	2228.00	2268.00	6839.000	6631.000	17.966	43.266	2521.000	22.000	21.000	1.251	0.400	67.080	0.918	0.913	-0.900	-0.900	4.270	4.290	0.753	0.683	-0.900	-1.600	8.370	7.080
JK03	2563.00	2382.00	7548.000	7746.000	20.239	43.460	2502.000	256.000	242.000	1.320	1.100	68.500	1.065	1.053	0.200	0.100	4.930	4.860	0.853	0.880	0.000	0.200	9.800	11.000
JK47	1901.00	1765.00	5431.000	5662.000	14.759	34.047	2042.000	32.000	31.000	1.265	1.200	64.510	0.953	0.920	-0.200	-0.400	4.030	4.010	0.748	0.804	-0.400	0.100	7.250	8.420
JK60	2874.00	2874.00	11423.000	10799.000	27.970	59.226	2943.000	1407.000	1327.000	1.343	0.200	88.240	1.161	1.184	0.300	0.400	5.740	5.870	0.927	0.949	-0.200	0.000	12.760	13.670
JK39	1760.00	1697.00	6077.000	6188.000	15.722	34.796	1852.000	310.000	293.000	1.040	-1.000	49.320	0.981	1.037	-0.200	0.200	4.000	4.120	0.667	0.653	-1.400	-1.500	6.180	5.770
JK67	1884.00	1917.00	6364.000	6168.000	16.333	35.775	2060.000	40.000	38.000	1.195	0.500	55.350	0.981	0.935	0.000	-0.400	4.400	4.150	0.779	0.755	-0.300	-0.500	7.630	7.620
JK65	2792.00	2721.00	7726.000	7379.000	20.618	45.714	2578.000	48.000	45.000	1.335	1.200	71.510	1.128	1.067	0.800	0.300	5.120	5.130	0.917	0.936	0.600	0.800	12.510	11.930
JK61	2420.00	2250.00	7875.000	7556.000	20.101	42.192	2465.000	0.000	0.000	1.255	0.200	65.370	1.152	1.197	0.700	1.100	5.150	5.040	0.960	0.916	0.700	0.400	10.350	8.850
JK02	2834.00	2455.00	8461.000	8502.000	22.252	48.166	2709.000	542.000	511.000	1.440	1.500	78.970	1.038	1.029	-0.200	-0.300	4.910	4.820	0.842	0.812	-0.500	-0.700	8.970	8.920
JK63	2711.00	2538.00	8332.000	8090.000	21.671	47.841	2874.000	87.000	82.000	1.359	1.200	75.140	1.122	1.118	0.600	0.500	5.760	5.860	0.909	0.890	0.300	0.200	12.620	13.250
JK37	2031.00	2021.00	5895.000	5724.000	15.671	36.845	2488.000	29.000	27.000	1.252	1.000	73.240	1.037	1.004	0.500	0.200	4.600	4.510	0.797	0.773	0.000	-0.200	9.450	8.570
JK54	3042.00	2946.00	8104.000	7784.000	21.876	46.873	2305.000	540.000	510.000	1.212	0.000	66.710	1.004	1.058	-0.200	0.200	4.680	4.760	0.902	0.916	0.300	0.400	9.630	10.250
JK64	1901.00	1844.00	6956.000	6836.000	17.537	39.202	2662.000	110.000	104.000	1.305	1.100	68.870	1.191	1.200	1.300	1.400	5.580	5.580	0.887	0.888	0.500	0.500	9.770	9.910
JK70	2085.00	1984.00	6104.000	5904.000	16.077	36.773	2089.000	109.000	103.000	1.170	0.200	59.400	1.126	1.099	1.000	0.800	4.480	4.410	0.743	0.716	-0.600	-0.800	7.440	7.250
JK77	3104.00	2772.00	8914.000	9316.000	24.106	52.714	3229.000	5.000	5.000	1.520	2.100	95.720	1.153	1.095	0.600	0.200	5.690	5.760	1.051	1.020	1.300	1.100	11.930	11.320
JK55	2210.00	2404.00	8041.000	7694.000	20.349	46.814	2770.000	1364.000	1286.000	1.300	0.000	67.080	1.023	0.999	-0.500	-0.700	5.080	5.290	0.880	0.870	-0.400	-0.500	11.710	10.100
JK71	2190.00	2316.00	7000.000	7159.000	18.665	42.530	2370.000	515.000	486.000	1.228	0.100	60.350	1.041	0.988	0.100	-0.300	4.700	4.490	0.884	0.863	0.200	0.000	9.780	9.680
JK43	2818.00	2701.00	8393.000	8358.000	22.270	47.597	2501.000	747.000	705.000	1.322	0.700	69.100	1.055	1.157	0.100	0.900	4.730	4.870	0.966	0.997	0.800	1.100	11.230	11.490
JK75	2250.00	2036.00	6484.000	5962.000	16.732	39.097	2324.000	203.000	191.000	1.148	-0.200	61.120	0.958	1.003	-0.200	0.100	4.920	5.060	0.697	0.760	-1.100	-0.600	7.410	8.390
JK40	1833.00	1743.00	4957.000	5200.000	13.733	32.814	1785.000	4.000	3.000	1.117	0.200	51.380	0.737	0.749	-1.300	-1.300	3.320	3.210	0.603	0.597	-1.400	-1.400	6.740	6.770
JK45	2650.00	2627.00	8508.000	8140.000	21.925	47.857	2945.000	1306.000	1232.000	1.364	0.600	81.780	1.091	1.135	0.200	0.500	6.380	6.760	0.837	0.943	-0.600	0.300	14.290	16.640
JK73	2444.00	2299.00	7624.000	7467.000	19.834	44.098	2607.000	129.000	122.000	1.154	-0.100	61.280	1.000	0.969	0.200	0.000	5.060	4.910	0.747	0.714	-0.600	-0.900	9.000	8.940
JK58	2624.00	2624.00	8909.000	8843.000	23.000	49.776	2580.000	976.000	921.000	1.180	-1.200	67.840	1.006	1.034	-0.600	-0.400	4.620	4.700	0.775	0.781	-1.300	-1.300	9.070	9.690
JK69	2442.00	2342.00	7424.000	7766.000	19.974	43.398	2640.000	1408.000	1328.000	1.318	0.500	71.690	0.953	0.942	-0.600	-0.700	4.970	4.960	0.782	0.758	-0.900	-1.100	8.960	8.530
JK72	2930.00	2930.00	9082.000	9221.000	24.163	57.044	2626.000	2528.000	2385.000	1.268	-0.400	72.090	1.012	1.013	-0.500	0.100	4.370	5.010	0.879	0.870	-0.400	-0.500	10.260	10.220
JK98	1758.00	1739.00	6546.000	6381.000	16.424	36.414	2035.000	685.000	646.000	1.169	-0.100	55.220	1.034	0.994	0.400	0.100	4.670	4.580	0.786	0.786	-0.300	-0.400	8.530	9.120
JK78	3707.00	3707.00	9553.000	9326.000	26.293	56.724	2721.000	1765.000	1665.000	1.319	0.200	74.730	1.108	1.067	0.300	0.000	5.320	5.450						

JK115	1750.00	1502.00	5070.000	4848.000	13.170	30.383	1958.500	54.000	51.000	1.127	-	57.660	0.963	0.997	-	-	4.080	4.240	0.680	0.735	-	-	-	-	-	-	5.290	5.960
JK25	1727.00	1756.00	5467.000	5592.000	14.542	34.797	2178.600	21.000	20.000	1.210	-	60.090	1.010	1.052	-	-	4.560	4.750	0.848	0.899	-	-	-	-	-	-	8.440	8.740
JK116	2398.00	2398.00	7287.000	7376.000	19.459	42.845	1923.400	1335.000	1260.000	1.060	-	51.130	1.041	0.981	-	-	4.090	3.960	0.809	0.735	-	-	-	-	-	-	7.770	6.900
JK76	1675.00	1715.00	5135.000	5297.000	13.822	33.231	1923.000	100.000	94.000	1.134	0.100	57.920	0.985	1.070	-0.200	0.400	3.610	3.990	0.838	0.907	0.200	-	-	0.800	0.800	5.470	7.170	
JK106	1612.00	1493.00	5508.000	5380.000	13.993	34.752	2049.000	0.000	0.000	1.160	0.300	58.440	0.902	0.878	-0.800	-1.000	4.220	4.240	0.716	0.737	-0.900	-0.700	-	-	6.600	6.790		
JK108	1864.00	1887.00	6266.000	5457.000	15.474	36.126	2193.000	43.000	40.000	1.093	-0.300	53.480	0.966	0.985	-0.400	-0.200	4.190	4.240	0.769	0.815	-0.500	-0.100	-	-	7.880	9.070		
JK110	1924.00	1862.00	6129.000	5834.000	15.749	37.827	2152.000	14.000	14.000	1.132	0.100	52.970	1.079	1.020	0.500	0.000	5.090	4.740	0.825	0.759	0.100	-	-	0.500	7.770	6.650		
JK59	1732.00	1735.00	5507.000	5793.000	14.767	33.898	2010.000	822.000	775.000	1.256	0.700	60.230	1.130	1.127	0.600	0.600	4.860	4.800	0.810	0.780	-0.400	-0.600	-	-	5.950	5.270		
JK32	1742.00	1619.00	5765.000	5732.000	14.858	32.802	1886.000	379.000	358.000	0.994	-1.100	43.090	0.935	0.999	-0.500	-0.100	3.970	4.090	0.797	0.841	-0.200	-	-	0.200	6.230	6.660		
JK29	1968.00	1754.00	5614.000	5756.000	15.092	35.891	2190.000	8.000	7.000	1.209	0.700	62.650	0.980	0.926	-0.200	-0.600	4.590	4.330	0.787	0.764	-0.200	-0.400	-	-	7.650	6.920		
JK62	1802.00	1691.00	6779.000	6424.000	16.696	37.515	2070.000	86.000	82.000	1.154	0.100	58.660	0.939	0.968	-0.600	-0.400	4.580	4.830	0.775	0.769	-0.400	-0.500	-	-	7.090	7.930		
JK107	1413.00	1366.00	4479.000	4392.000	11.650	28.352	1778.000	237.000	224.000	1.145	0.300	48.900	0.855	0.822	-1.000	-1.200	3.730	3.720	0.656	0.680	-1.300	-1.000	-	-	5.240	5.730		
JK41	2614.00	2572.00	9156.000	9246.000	23.588	52.211	2600.000	1104.000	1041.000	1.172	-1.100	65.170	0.967	0.960	-1.300	-1.300	4.690	4.480	0.800	0.795	-1.300	-1.400	-	-	9.990	9.770		
JK102	1483.00	1431.00	5293.000	5115.000	13.322	30.813	1767.000	180.000	170.000	1.030	-0.800	47.910	0.840	0.844	-1.200	-1.200	4.180	4.060	0.662	0.660	-1.300	-1.400	-	-	6.540	6.500		
JK114	2002.00	2068.00	6298.000	6124.000	16.492	37.781	1832.000	126.000	118.000	1.049	-0.700	49.170	0.790	0.832	-1.600	-1.300	3.750	3.830	0.647	0.649	-1.500	-1.500	-	-	7.480	6.010		
JK05	2771.00	2567.00	7637.000	7447.000	20.422	43.413	2507.000	263.000	248.000	1.384	1.700	75.850	1.119	1.185	0.600	1.000	4.990	5.130	0.961	0.982	1.000	1.100	-	-	11.840	11.860		
JK109	1863.00	1661.00	6635.000	6457.000	16.616	39.915	2621.000	99.000	93.000	1.362	1.400	86.780	0.916	0.891	-0.800	-1.000	5.280	5.200	0.770	0.776	-0.600	-0.700	-	-	8.730	9.760		
JK87	1420.00	1457.00	5977.000	6033.000	14.887	34.533	2171.000	65.000	62.000	1.128	0.000	62.370	0.910	0.965	-0.600	-0.200	3.840	4.140	0.713	0.698	-0.900	-1.000	-	-	7.230	7.030		
JK97	1343.00	1344.00	4650.000	4508.000	11.845	29.833	1949.000	153.000	144.000	1.124	0.200	54.990	1.020	0.966	0.300	-0.100	4.500	4.250	0.822	0.797	0.300	0.000	-	-	8.240	7.680		
JK101	1864.00	1828.00	6222.000	5903.000	15.821	37.142	2067.000	26.000	24.000	1.172	0.300	55.070	0.859	0.815	-1.000	-1.300	4.260	4.300	0.711	0.727	-0.900	-0.700	-	-	7.460	7.430		
JK28	1872.00	1803.00	6005.000	6044.000	15.724	36.884	2230.000	829.000	782.000	1.115	-0.500	56.150	0.917	0.947	-0.800	-0.500	4.270	4.560	0.750	0.712	-0.800	-1.100	-	-	7.610	7.100		
JK52	1676.00	1661.00	5789.000	5691.000	14.817	31.547	2084.000	519.000	490.000	1.341	1.700	61.100	1.046	1.022	0.300	0.200	4.600	4.500	0.809	0.790	-0.100	-0.200	-	-	6.480	6.570		
JK112	2172.00	2320.00	7173.000	7383.000	19.048	41.703	2416.000	550.000	519.000	1.221	0.200	65.530	0.876	0.863	-1.100	-1.200	4.320	4.190	0.693	0.708	-1.400	-1.200	-	-	8.440	8.300		
JK53	2440.00	2302.00	6776.000	6784.000	18.302	41.510	2154.000	469.000	443.000	1.312	1.000	61.610	0.907	0.912	-0.800	-0.800	4.080	4.100	0.753	0.722	-0.800	-1.100	-	-	7.630	7.900		
JK113	1361.00	1235.00	4264.000	4224.000	11.084	28.069	1742.000	188.000	177.000	1.025	-0.600	48.370	0.812	0.798	-0.900	-1.000	3.400	3.400	0.658	0.695	-1.000	-0.700	-	-	6.430	6.900		
JK103	2651.00	2588.00	7675.000	7832.000	20.746	45.683	2671.000	551.000	520.000	1.421	1.900	68.950	1.124	1.118	0.900	0.800	5.460	5.180	0.916	0.929	0.700	0.800	-	-	10.260	10.580		
JK95	1966.00	1879.00	6787.000	6586.000	17.218	40.157	2105.000	541.000	511.000	1.194	0.100	61.720	0.894	0.912	-0.500	-0.400	3.980	4.000	0.686	0.702	-1.100	-1.000	-	-	6.950	8.430		
JK117	1584.00	1512.00	6147.000	6191.000	15.434	35.735	2364.000	218.000	206.000	1.138	-0.100	59.530	0.878	0.901	-0.800	-0.600	4.080	4.250	0.697	0.699	-1.000	-1.000	-	-	7.050	7.240		
JK118	1305.00	1263.00	5520.000	5102.000	13.190	33.075	1882.000	204.000	192.000	0.953	-1.400	46.230	0.818	0.835	-1.100	-1.000	3.790	3.910	0.598	0.621	-1.700	-1.500	-	-	6.160	6.690		
JK119	1717.00	1617.00	5650.000	5634.000	14.618	33.409	2140.000	185.000	174.000	1.273	1.200	60.930	0.942	0.955	-0.200	-0.100	4.350	4.360	0.745	0.799	-0.500	0.000	-	-	7.210	7.890		
JK120	1822.00	1809.00	5728.000	5662.000	15.021	36.175	2220.000	224.000	211.000	1.231	0.800	69.040	0.858	0.853	-0.800	-0.800	4.080	3.940	0.681	0.700	-1.000	-0.900	-	-	6.310	6.370		
JK121	1351.00	1447.00	5862.000	5875.000	14.535	33.853	1853.000	559.000	528.000	1.159	0.200	60.420	0.760	0.777	-1.500	-1.400	3.830	3.930	0.636	0.623	-1.400	-1.600	-	-	6.980	6.630		
JK20	2615.00	2457.00	7113.000	7163.000	19.348	42.472	2068.500	850.000	801.000	1.212	-	55.680	1.023	0.973	-	-	4.550	4.390	0.937	0.835	-	-	-	-	10.730	9.000		
JK21	1804.00	1716.00	5467.000	5327.000	14.314	31.542	2101.700	222.000	210.000	1.281	-	58.150	0.842	0.835	-	-	3.950	3.910	0.670	0.681	-	-	-	-	7.430	7.800		
JK51	2313.00	2154.00	7283.000	7054.000	18.804	40.015	2294.000	545.000	514.000	1.198	-0.100	61.610	1.027	1.132	-0.400	0.400	4.430	4.770	0.819	0.838	-0.600	-0.400	-	-	9.500	9.490		
JK88	2371.00	2324.00	8077.000	7925.000	20.697	41.976	2278.000	612.000	578.000	1.323	0.700	58.510	1.203	1.155	0.700	0.400	5.250	5.100	0.924	0.913	0.200	0.100	-	-	8.980	8.380		
JK44	2113.00	1927.00	7064.000	6625.000	17.729	39.767	2289.000	0.000	0.000	1.023	-1.300	50.700	0.832	0.815	-1.600	-1.700	4.150	4.150	0.657	0.698	-1.800	-1.400	-	-	6.590	7.710		
JK35	2110.00	2108.00	6396.000	6217.000	16.831	35.978	2060.000	180.000	170.000	1.105	-0.500	55.400	0.850	0.844	-1.400	-1.400	3.900	3.850	0.631	0.645	-1.900	-1.700	-	-	6.200	6.040		
JK79	1678.00	1541.00	4728.000	4597.000	12.544	27.965	1774.000	79.000	74.000	1.007	-0.700	42.300	0.930	0.926	-0.400	-0.400	3.960	3.830	0.659	0.652	-1.200	-1.200	-	-	6.250	6.150		
JK89	1457.00	1434.00	4193.000	4419.000	11.503	27.318	1764.000	36.000	34.000	1.096	0.000	51.050	0.815	0.816	-1.200	-1.200	3.330	3.380	0.570	0.605	-1.900	-1.600	-	-	5.570	5.780		
JK91	1627.00	1627.00	5431.000	5349.000	14.034	30.994	2034.000	416.000	393.000	1.268	1.000	67.900	0.913	0.917	-0.700	-0.700	3.970	3.940	0.791	0.803	-0.300	-0.200	-	-	8.520	8.940		
JK80	2773.00	2705.00	8356.000	8247.000	22.081	48.391	2634.000	357.000	337.000	1.204	-0.200	75.220	0.910	0.900	-1.200	-1.200	4.580	4.600	0.821	0.837	-0.600	-0.400	-	-	11.360	11.670		
JK83	2258.00	1944.00	7547.000	7503.000	19.252	38.883	2179.000	324.000	306.000	1.349	1.300	64.720	1.087	1.074	0.300	0.200	4.920	4.970	0.805	0.809	-0.500	-0.500	-	-	8.380	8.430		
JK11	2001.00	1862.00	5778.000	5527.000	15.168	33.688	2051.000	72.000	68.000	1.191	0.400	47.110	0.890	0.936	-0.800	-0.500	4.140	4.130	0.671	0.704	-1.000							

ID	TH_BMD_left	TH_BMD_rt	TH_BMD_left_Z	TH_BMD_rt_Z	TH_BMC_left	TH_BMC_rt	Cbpaq	pbpaq	tbpaq	US_BORN	US_LIFE_Y	CURRENT_oc	CURRENT_oc_YR	MENARCHE	ca_1day	totalPAMET_ipaq	PAlevel	total_vitD_day	sun_exp_sc	BMC_4	Vbmd_4	Trab_BMC_4	Trab_vBMD_4	Area_4	Trab_Area_4	PC_4	BSI_total_4	
JK30	1.017	1.067	.	.	26.350	28.900	0.616	133.155	66.88500	1.0	18.0	0.0	0.0	0.0	12.000	240.714	1128.000	2.00	58.629	40.000	250.800	315.600	169.780	263.700	794.560	643.840	99.924	79.141
JK04	0.891	0.911	.	.	23.890	25.220	42.390	72.992	57.69100	1.0	19.0	0.0	0.0	0.0	14.000	1845.714	2218.500	2.00	81.486	16.000	250.860	268.400	176.910	228.200	934.560	775.360	108.370	67.324
JK38	1.092	1.105	.	.	32.100	32.350	0.968	55.131	28.05000	1.0	19.0	1.0	9.0	11.000	675.714	1039.500	2.00	70.457	23.000	336.090	315.500	245.900	278.600	1065.280	882.560	115.701	106.038	
JK12	0.967	1.000	.	.	25.010	26.300	0.968	51.800	26.38400	1.0	19.7	0.0	0.0	0.0	12.000	880.000	2553.000	2.00	68.114	24.000	252.950	300.300	168.240	247.000	842.240	681.120	102.878	75.953
JK26	0.983	0.941	.	.	30.030	29.100	0.572	10.897	5.79400	1.0	19.8	0.0	0.0	0.0	14.000	829.286	1607.000	2.00	119.314	14.000	298.680	308.900	196.790	252.000	967.040	780.800	110.237	92.274
JK13	1.103	1.088	0.700	0.600	35.330	34.690	20.474	108.270	64.37200	1.0	20.8	1.0	0.0	0.0	13.000	817.143	3948.000	1.00	211.629	27.000	364.060	383.600	225.300	305.800	949.120	736.800	109.211	139.662
JK22	0.901	0.901	-0.500	-0.500	23.480	23.590	13.180	65.047	39.11300	1.0	20.8	0.0	0.0	0.0	11.000	957.857	1828.500	2.00	80.143	22.000	258.930	288.600	187.130	252.000	897.120	742.560	106.177	74.721
JK10	1.048	1.003	0.600	0.200	28.670	27.860	2.352	30.363	46.35700	1.0	21.6	0.0	0.0	0.0	15.000	879.286	7716.000	1.00	127.657	40.000	264.230	295.300	166.030	231.300	894.880	717.760	106.044	78.035
JK16	1.254	1.236	1.500	1.300	37.990	37.170	9.181	22.354	15.76700	1.0	21.8	1.0	0.0	0.0	13.000	378.571	3411.000	1.00	41.171	29.000	357.500	317.200	267.740	284.800	1127.040	940.160	119.008	113.398
JK15	1.028	1.005	0.300	0.100	30.590	29.810	5.069	30.590	17.88800	1.0	21.9	1.0	6.0	13.000	576.429	3424.000	1.00	216.814	36.000	270.470	289.600	185.750	243.900	933.920	764.800	108.333	78.326	
JK09	1.026	1.024	0.200	0.200	30.350	30.120	31.359	274.933	153.14600	1.0	22.9	1.0	3.0	14.000	746.429	868.000	2.00	28.914	26.000	284.530	295.200	207.910	260.200	964.000	799.040	110.678	84.006	
JK19	1.086	1.083	0.500	0.500	34.370	34.520	6.442	25.518	15.98000	1.0	24.1	1.0	0.5	15.000	887.857	1044.500	2.00	1171.171	19.000	260.830	344.400	165.640	277.400	975.280	920.120	97.551	89.822	
JK07	0.960	0.991	-0.300	0.000	29.900	31.760	2.909	178.915	90.91000	1.0	25.5	1.0	0.0	0.0	13.000	944.286	4573.500	1.00	984.686	21.000	328.160	309.600	225.270	259.700	1060.000	867.360	115.414	101.603
JK24	0.972	0.958	0.000	-0.100	27.030	26.050	1.602	48.063	24.83200	1.0	25.7	1.0	0.0	0.0	12.000	573.571	598.500	3.00	80.257	14.000	216.640	268.800	137.980	210.100	808.080	656.640	100.645	58.242
JK49	1.051	1.041	0.200	0.100	33.870	33.160	5.450	36.689	21.07000	1.0	26.0	0.0	0.0	0.0	13.000	497.857	5136.000	1.00	613.657	11.000	320.800	304.900	217.320	253.800	1052.320	856.120	114.995	97.828
JK42	1.180	1.182	0.600	0.600	35.180	31.500	2.790	72.879	37.83400	1.0	26.1	0.0	0.0	0.0	13.000	1444.286	1730.000	2.00	42.400	15.000	338.000	356.000	211.630	286.500	949.440	738.720	109.229	120.328
JK08	1.002	0.990	0.000	-0.100	30.840	29.950	4.157	102.726	53.44000	1.0	26.4	0.0	0.0	0.0	13.000	544.286	3561.000	1.00	624.229	25.000	293.800	257.600	204.480	215.200	1140.480	950.240	119.715	75.680
JK01	1.074	1.028	0.100	-0.300	36.280	34.720	2.860	125.069	63.96500	1.0	26.6	1.0	8.0	12.000	649.286	8115.000	1.00	1012.000	14.000	338.290	286.300	239.700	243.800	1181.760	983.240	121.862	96.866	
JK46	0.938	0.925	-0.600	-0.700	28.240	27.010	11.760	34.795	23.27800	1.0	27.3	1.0	0.6	11.000	474.286	1380.000	2.00	80.286	11.000	281.920	298.300	175.050	233.100	943.200	751.040	108.870	84.267	
JK03	1.100	1.104	0.700	0.700	32.650	33.960	3.515	58.361	30.83800	0.0	5.0	0.0	0.0	0.0	13.000	1322.857	4327.500	1.00	870.457	40.000	327.390	335.300	228.720	236.900	976.320	795.360	110.765	109.764
JK47	0.944	0.952	-0.100	0.000	26.460	27.180	4.682	11.384	8.03900	1.0	28.3	1.0	0.0	0.0	10.000	1218.571	1992.000	2.00	135.143	9.000	256.490	279.300	179.320	236.900	918.240	756.960	107.419	71.631
JK60	1.208	1.218	0.800	0.900	40.860	41.930	1.073	15.201	8.14000	1.0	29.4	0.0	0.0	0.0	11.000	782.143	4312.500	1.00	150.714	24.000	408.430	339.900	257.830	271.500	1201.600	949.760	122.881	138.823
JK39	0.896	0.940	-0.700	-0.300	23.530	24.090	3.212	21.708	12.46000	0.0	2.0	0.0	0.0	0.0	14.000	746.429	1746.000	2.00	55.314	28.000	238.050	271.500	151.070	212.400	876.960	711.360	104.977	64.643
JK67	1.026	0.991	0.500	0.200	27.850	26.830	3.168	35.882	19.52500	1.0	29.8	1.0	0.0	0.0	13.000	1660.714	4809.000	1.00	692.371	14.000	261.610	325.100	165.200	260.300	804.640	634.560	100.555	85.042
JK65	1.079	1.090	0.600	0.700	34.740	34.940	0.485	67.372	33.92800	1.0	30.9	1.0	8.0	12.000	1577.857	3132.000	1.00	170.143	6.000	372.550	340.800	262.830	295.000	1093.280	891.040	117.212	126.979	
JK61	1.152	1.149	1.000	0.900	33.620	31.810	2.440	38.050	20.25000	1.0	31.1	0.0	0.0	0.0	12.000	1160.714	2874.000	2.00	247.286	13.000	334.360	379.100	214.000	310.600	882.080	688.960	105.283	126.770
JK02	1.055	1.053	0.000	0.000	32.410	32.170	0.000	156.143	78.07200	1.0	31.3	1.0	0.0	0.0	10.000	810.714	2412.600	2.00	138.857	41.000	422.960	353.200	294.680	303.400	1197.440	971.360	122.668	149.381
JK63	1.109	1.080	0.700	0.400	37.510	37.610	5.166	62.809	33.98700	1.0	31.4	1.0	0.0	0.0	11.000	597.143	5313.000	1.00	2111.686	18.000	331.680	263.100	233.910	222.700	1260.640	1050.400	125.864	87.264
JK37	1.034	0.984	0.600	0.200	30.630	29.810	0.506	141.831	71.16800	1.0	31.8	1.0	0.0	0.0	12.000	1367.857	3397.500	1.00	1182.971	27.000	288.950	295.200	192.800	241.200	978.720	796.800	110.901	85.289
JK54	1.084	1.088	0.500	0.500	32.040	32.420	6.494	40.741	23.61700	1.0	31.9	1.0	0.0	0.0	12.000	828.571	2980.000	2.00	726.057	9.000	311.380	350.700	216.420	304.100	887.840	711.680	105.626	109.196
JK64	1.163	1.172	1.400	1.400	35.150	35.330	4.330	3.808	4.06900	1.0	32.0	1.0	0.0	0.0	14.000	452.857	1906.500	2.00	778.000	4.000	342.370	322.000	215.860	254.200	1063.360	849.280	115.597	110.253
JK70	0.986	0.977	0.100	0.000	28.270	27.520	0.290	56.130	28.21000	1.0	32.6	1.0	16.7	13.000	886.429	2118.000	2.00	5174.029	7.000	253.500	282.500	154.530	215.300	897.280	717.760	106.186	71.609	
JK77	1.227	1.194	1.400	1.100	37.740	36.730	7.900	22.090	15.00000	1.0	34.2	0.0	0.0	0.0	10.000	668.571	2997.000	2.00	146.943	16.000	402.240	349.700	284.030	301.800	1150.240	940.960	120.226	140.663
JK55	1.067	1.076	-0.100	0.000	35.490	34.470	2.480	34.980	18.73000	1.0	34.5	1.0	0.0	0.0	12.000	773.286	1328.000	2.00	2097.114	6.000	334.770	337.700	204.320	261.600	991.200	781.120	111.605	113.038
JK71	1.091	1.075	0.600	0.500	31.150	31.010	5.600	22.013	13.81000	1.0	35.8	0.0	0.0	0.0	13.000	1275.000	3846.000	1.00	1168.343	9.000	327.990	334.900	223.800	283.100	979.360	790.560	110.937	109.843
JK43	1.173	1.204	1.100	1.400	34.500	34.350	2.052	38.961	20.50600	1.0	35.9	0.0	0.0	0.0	11.000	453.571	1977.000	2.00	5115.714	16.000	273.780	311.100	173.120	246.300	880.000	702.880	105.159	85.169
JK75	0.978	1.040	-0.100	0.400	29.370	31.130	0.880	16.280	8.58000	1.0	36.4	0.0	0.0	0.0	14.000	814.286	2736.000	2.00	200.514	9.000	280.690	277.500	188.860	227.800	1011.520	828.860	112.744	77.893
JK40	0.737	0.728	-1.500	-1.500	21.830	21.200	1.098	13.228	7.16300	1.0	37.8	1.0	3.0	13.000	642.857	1965.000	2.00	41.771	12.000	195.550	263.100	111.450	187.500	743.360	594.400	96.651	51.457	
JK45	1.053	1.142	-0.100	0.600	40.840	44.470	1.930	20.191	11.06000	1.0	38.4	1.0	0.0	0.0	11.000	666.429	1597.500	2.00	2124.457	28.000	325.520	299.900	197.860	228.300				

ID	BSI_trab_4	BMC_38	vBMD_38	Area_38	C_BMC_38	C_vBMD_38	C_Area_38	C_Tk_38	PC_38	EC_38	iPOLAR_38	SSI_38	BMC_66	Vbmd_66	Area_66	C_BMC_66	C_vBMD_66	C_Area_66	C_Tk_66	PC_66	EC_66	iPOLAR_66	SSI_66	MCSA	msl_dens	serumVITD
JK30	44.771	284.730	953.700	298.560	276.560	1213.000	228.000	5.009	61.252	29.777	14985.16	1193.667	309.920	648.300	478.080	263.240	1149.700	228.960	3.431	77.510	55.951	30051.55	1822.143	6847.52	79.10	33.99
JK04	40.377	290.050	849.500	341.440	263.690	1146.100	230.080	4.471	65.503	37.408	17773.81	1291.352	318.650	676.300	471.200	289.480	1130.800	256.000	3.970	76.950	52.003	31092.97	1827.102	5453.12	77.20	31.89
JK38	68.503	355.820	994.100	357.920	347.780	1213.600	286.560	5.908	67.065	29.946	22223.06	1549.859	387.010	744.700	519.680	362.420	1170.600	309.600	4.684	80.811	51.380	40610.62	2465.283	6615.04	78.90	55.30
JK12	41.554	302.190	974.100	310.240	290.700	1200.000	242.240	5.285	62.439	29.232	16340.84	1227.282	326.020	768.000	424.480	302.410	1153.900	262.080	4.434	73.035	45.175	26608.52	1790.045	5654.40	80.40	20.33
JK26	49.584	336.950	906.200	371.840	315.290	1163.300	271.040	5.215	68.357	35.591	23079.32	1534.942	364.470	695.300	529.920	341.760	1124.800	303.840	4.505	81.604	53.301	40742.68	2300.708	6835.18	78.80	20.43
JK13	68.901	365.280	964.500	378.720	347.200	1191.700	291.360	5.706	68.986	33.133	23915.57	1713.860	405.930	775.900	523.200	366.710	1165.200	314.720	4.759	81.085	51.184	41746.90	2462.697	5748.96	80.80	54.97
JK22	47.156	274.440	928.700	295.520	266.830	1203.200	221.760	4.853	60.939	30.445	14620.39	1132.309	313.200	656.000	477.440	282.120	1160.000	243.200	3.693	77.458	54.254	31388.04	1988.657	8091.04	78.40	34.55
JK10	38.400	310.940	1000.700	310.720	302.830	1205.500	251.200	5.592	62.487	27.349	17832.53	1274.272	323.620	719.000	450.080	306.530	1171.700	261.600	4.224	75.206	48.667	31954.04	1877.701	5831.04	78.10	39.31
JK16	76.257	394.610	966.800	408.160	380.050	1176.500	323.040	6.193	71.618	32.705	26917.26	1802.352	405.530	741.300	547.040	362.790	1132.600	320.320	4.701	82.911	53.376	43341.58	2454.363	5493.60	80.40	28.56
JK15	45.124	310.080	922.000	336.320	296.400	1202.900	246.400	4.997	65.010	33.615	20058.54	1299.547	333.830	659.200	506.400	303.860	1160.100	261.920	3.875	79.772	55.428	36515.76	1997.814	5598.33	78.60	27.40
JK09	54.098	330.270	936.600	352.640	315.000	1171.200	268.960	5.434	66.569	32.428	20272.85	1488.079	326.760	654.100	514.880	305.720	1124.600	271.840	4.006	80.437	55.264	35874.20	2195.301	4789.12	81.10	42.04
JK19	45.949	283.710	949.300	298.880	267.910	1195.200	224.160	4.877	61.285	30.642	15332.14	1186.294	327.560	715.800	457.600	294.300	1148.200	256.320	4.065	75.831	50.293	30251.86	1911.767	4828.48	77.30	30.31
JK07	58.498	372.700	895.200	416.320	345.970	1192.700	290.080	5.173	72.330	39.829	27252.95	1852.957	386.940	605.700	638.880	332.940	1131.500	294.240	3.787	89.601	65.809	50996.31	2721.826	6738.72	78.70	38.35
JK24	28.985	249.280	948.800	262.720	235.060	1204.200	195.200	4.509	57.458	29.129	12672.49	956.966	297.080	685.400	433.440	265.050	1181.600	224.320	3.587	73.802	51.263	27899.77	1621.641	4811.52	79.90	17.62
JK49	55.149	320.660	936.500	342.400	305.350	1200.300	254.400	5.147	65.595	33.254	20315.18	1380.230	324.680	634.300	511.840	287.210	1155.900	248.480	3.608	80.200	57.528	34670.28	1976.036	7094.56	78.70	22.43
JK42	60.636	312.380	973.800	320.800	302.780	1205.300	251.200	5.398	63.492	29.574	18086.23	1331.022	359.340	747.100	480.960	332.490	1178.700	282.080	4.417	77.743	49.992	51550.63	2767.223	6315.36	79.00	25.36
JK08	44.007	379.100	908.800	417.120	368.330	1202.700	306.240	5.582	72.399	37.328	28605.37	1814.687	410.420	648.300	633.120	376.680	1149.500	327.680	4.336	89.197	61.954	54002.97	2768.546	6916.80	78.70	46.06
JK01	58.430	455.870	981.100	464.640	433.680	1188.800	364.800	6.524	76.412	35.421	36045.01	2205.753	451.570	699.100	683.920	417.220	1147.700	363.520	4.858	90.094	59.571	58451.28	3184.233	8892.96	78.60	37.63
JK46	40.808	368.140	900.500	408.800	356.800	1198.300	297.760	5.462	71.674	37.355	26771.45	1847.053	389.950	680.000	573.440	363.440	1168.500	311.040	4.371	84.889	57.423	62683.24	3378.083	6665.76	78.10	17.59
JK03	65.787	369.770	1033.600	357.760	357.790	1210.700	295.520	6.220	67.050	27.967	22626.28	1577.980	396.530	801.300	494.880	375.190	1184.900	316.640	5.019	78.860	47.327	39846.16	2286.537	6423.52	79.60	128.59
JK47	42.482	311.030	921.700	337.440	293.010	1204.000	243.360	4.892	65.118	34.384	20050.04	1382.794	310.870	633.500	490.720	264.120	1156.800	228.320	3.359	78.528	57.423	46882.54	2654.882	2555.84	78.70	18.58
JK60	70.009	419.280	1011.400	414.560	396.790	1184.900	334.880	6.451	72.177	31.643	29498.04	1847.094	433.810	747.500	580.320	374.670	1124.700	333.120	4.721	85.396	55.735	23176.06	1591.738	4506.24	79.10	25.90
JK39	32.092	289.850	907.100	319.520	282.980	1208.900	234.080	4.870	63.366	32.767	17363.50	1282.846	319.810	676.000	473.120	295.660	1152.000	256.640	3.971	77.106	52.157	40610.62	2465.283	7603.20	77.80	29.85
JK67	42.995	307.280	1002.900	306.400	299.090	1222.600	244.640	5.442	62.051	27.859	17148.80	1237.092	328.820	756.700	435.840	305.700	1175.800	260.000	4.297	74.006	47.007	30372.77	1883.639	6952.64	78.00	37.82
JK65	77.543	365.050	996.300	366.400	346.710	1220.800	284.000	5.678	67.855	32.179	23151.52	1637.059	397.800	758.200	524.640	366.870	1194.800	307.040	4.600	81.196	52.292	39296.67	2284.072	6591.04	77.00	21.84
JK61	66.466	360.110	977.700	368.320	350.210	1193.500	293.440	5.946	68.033	30.675	22907.18	1575.056	384.190	778.300	493.600	361.600	1129.400	320.160	5.104	78.758	46.685	50340.78	2800.169	6058.24	79.20	21.98
JK02	89.415	385.560	1007.900	382.560	369.480	1206.500	306.240	6.106	69.335	30.969	26165.76	1672.352	385.560	1007.900	382.560	369.480	1206.500	306.240	6.106	69.335	30.969	53840.90	2733.358	6599.23	79.00	14.07
JK63	52.095	379.570	909.300	417.440	367.380	1224.000	300.160	5.417	72.427	38.390	29333.70	1943.353	427.330	637.900	669.920	382.390	1176.100	325.120	4.126	91.752	65.825	28255.80	1621.435	8906.88	76.00	41.34
JK37	46.356	347.830	999.000	348.160	326.910	1206.100	271.040	5.573	66.145	31.131	20172.16	1500.214	356.990	680.000	524.960	324.630	1187.200	273.440	3.979	81.221	56.220	38260.10	2326.713	7412.64	77.00	36.97
JK54	65.814	342.260	974.600	351.200	327.070	1163.500	281.120	5.850	66.433	29.676	21091.43	1397.305	381.600	793.400	480.960	356.930	1153.500	309.440	4.984	77.743	46.426	36002.42	2045.064	6652.18	78.80	30.98
JK64	54.878	316.130	894.000	353.600	305.870	1197.800	255.360	5.017	66.659	35.136	19630.80	1518.895	363.880	685.200	531.040	325.460	1153.800	282.080	4.099	81.690	55.933	61039.36	3098.804	5991.52	78.80	81.54
JK70	33.271	272.140	948.100	287.040	259.480	1224.000	212.000	4.671	60.059	30.708	13792.94	1098.699	312.990	691.200	452.800	282.140	1182.700	238.560	3.747	75.432	51.887	29823.18	1878.088	7283.04	78.10	22.05
JK77	85.706	439.960	968.600	454.240	417.670	1203.000	347.200	6.187	75.552	36.676	34529.87	2131.636	465.780	654.500	711.680	394.470	1138.200	346.560	4.270	94.569	67.736	66725.66	3325.658	7665.92	78.30	16.30
JK55	53.456	321.740	878.500	366.240	304.850	1219.000	250.080	4.716	67.840	38.206	21577.72	1580.456	355.090	648.900	547.200	315.470	1169.400	269.760	3.800	82.924	59.046	35110.63	2079.920	6745.44	77.60	17.49
JK71	63.360	318.050	991.900	320.640	310.540	1195.900	259.680	5.698	63.477	27.678	16999.84	1337.285	337.290	719.000	469.120	302.180	1137.000	265.760	4.174	76.780	50.552	31750.31	1983.155	3172.32	77.90	19.04
JK43	42.639	325.520	961.000	338.720	315.220	1170.600	269.280	5.682	65.242	29.540	19581.56	1346.392	368.120	733.200	502.080	333.920	1128.100	296.000	4.543	79.431	50.889	36014.98	2075.455	6136.80	79.70	18.14
JK75	43.017	382.930	959.200	399.200	373.330	1209.600	308.640	5.904	70.827	33.734	26081.41	1761.614	380.660	621.700	612.320	324.590	1134.600	286.080	3.770	87.719	64.029	46106.26	2684.228	6042.56	77.20	25.54
JK40	20.897	248.370	986.800	251.680	239.300	1248.400	191.680	4.580	56.238	27.459	10764.15	950.831	267.430	726.400	368.160	245.320	1206.300	203.360	3.583	68.018						

JK115	58.355	231.220	955.800	241.920	224.240	1196.800	187.360	4.608	55.137	26.184	9738.42	884.872	239.980	692.500	346.560	218.990	1169.800	187.200	3.381	65.992	44.750	16684.32	1254.779	8412.16	78.80	24.37
JK25	56.263	307.340	875.500	351.040	296.910	1205.800	246.240	4.795	66.418	36.290	19684.13	1480.387	352.190	759.000	464.000	330.120	1182.400	279.200	4.483	76.360	48.190	33234.43	2106.316	6912.48	81.00	21.95
JK116	71.498	275.340	870.500	316.320	262.460	1153.600	227.520	4.718	63.048	33.405	16756.06	1214.845	295.310	698.900	422.560	269.030	1133.000	237.440	3.921	72.870	48.232	26511.09	1715.865	5989.28	78.20	26.63
JK76	43.655	263.470	1012.700	260.160	253.490	1202.700	210.720	5.133	57.178	24.926	11410.52	936.422	272.340	872.400	312.160	260.470	1178.800	220.960	4.580	62.632	33.853	16138.59	1217.599	5796.64	77.40	30.40
JK106	59.511	255.940	857.200	298.560	246.620	1198.600	205.760	4.314	61.252	34.149	13933.02	1152.159	304.480	634.500	479.840	282.180	1156.500	244.000	3.694	77.652	54.439	29608.41	1974.772	5457.92	79.50	17.88
JK108	59.387	308.550	928.500	332.320	295.710	1190.100	248.480	5.119	64.622	32.459	18157.80	1307.705	332.560	735.000	452.480	293.530	1157.400	253.600	4.045	75.406	49.992	30820.02	1844.485	5636.00	78.10	27.59
JK110	76.520	370.070	1018.500	363.360	362.480	1188.600	304.960	6.443	67.573	27.090	23756.15	1595.847	376.280	788.100	477.440	356.790	1162.000	307.040	4.963	77.458	46.274	35566.23	2184.322	6904.16	77.50	46.03
JK59	32.707	252.490	931.600	271.040	239.430	1183.900	202.240	4.609	58.361	29.404	12365.20	1036.206	284.540	729.100	390.240	254.150	1150.200	220.960	3.805	70.028	46.122	33715.03	2024.717	8881.12	76.70	12.00
JK32	47.602	295.230	947.700	311.520	288.540	1210.300	238.400	5.134	62.567	30.313	17486.55	1249.039	321.160	789.000	407.040	306.850	1181.700	259.680	4.534	71.519	43.032	27324.82	1768.532	7393.28	79.40	21.52
JK29	39.145	304.770	917.500	332.160	296.190	1223.500	242.080	4.928	64.607	33.645	18685.27	1390.248	348.760	701.800	496.960	327.420	1178.100	277.920	4.227	79.025	52.465	35988.86	2259.210	6979.04	78.80	21.04
JK62	51.938	316.780	998.900	317.120	308.920	1218.900	253.440	5.545	63.127	28.288	18171.71	1253.729	340.110	863.700	393.760	325.590	1184.500	274.880	5.044	70.343	38.651	40159.23	2215.802	6071.84	80.30	13.12
JK107	42.096	224.700	993.200	226.240	217.280	1209.300	179.680	4.636	53.320	24.189	9154.90	771.091	262.250	771.000	340.160	244.550	1184.800	206.400	3.880	65.380	40.999	18153.18	1284.138	4888.96	79.20	21.70
JK41	66.002	368.980	888.700	415.200	350.080	1189.800	294.240	5.291	72.233	38.988	29184.51	1813.858	401.300	663.300	604.960	361.130	1131.400	319.200	4.339	87.190	59.925	19838.18	1419.914	11399.68	78.00	18.67
JK102	44.143	243.960	850.400	286.880	234.320	1205.300	194.400	4.130	60.042	34.090	13706.63	1074.590	251.940	549.600	458.400	216.650	1154.400	187.680	2.797	75.897	58.326	24516.74	1647.535	6110.40	79.70	18.75
JK114	45.852	308.980	942.500	327.840	289.420	1181.500	244.960	5.079	64.185	32.272	18503.53	1322.220	306.120	697.800	438.720	280.120	1145.800	244.480	3.954	74.250	49.405	29557.39	1720.315	5446.72	77.70	29.22
JK05	63.658	384.890	1023.200	376.160	375.520	1175.300	319.520	6.696	68.753	26.679	24684.03	1584.394	389.490	773.500	503.520	366.280	1149.200	318.720	4.990	79.545	48.190	39026.05	2319.694	7623.52	78.70	54.40
JK109	57.743	321.250	787.100	408.160	307.270	1205.500	254.880	4.413	71.618	43.888	26231.88	1739.692	369.290	528.900	698.240	335.630	1160.200	289.280	3.499	93.671	71.688	56566.77	3036.488	5309.28	78.80	19.81
JK87	59.245	308.070	865.400	356.000	298.980	1208.100	243.840	4.670	66.885	37.543	21666.43	1459.159	342.460	629.500	544.000	310.000	1176.400	263.520	3.710	82.681	59.368	41236.19	2275.224	7928.96	83.10	25.45
JK97	70.424	265.620	936.300	283.680	254.510	1213.000	213.440	4.774	59.706	29.710	14017.34	1134.570	297.760	755.600	394.080	278.420	1193.500	233.280	4.046	70.372	44.952	25284.01	1581.734	6480.96	78.60	21.39
JK101	48.681	292.330	850.600	343.680	283.280	1197.100	236.640	4.622	65.718	36.676	19116.87	1348.534	325.400	649.600	500.960	305.290	1157.800	263.680	3.937	79.343	54.605	35973.11	2083.028	5321.12	82.90	30.56
JK28	55.876	295.600	839.000	352.320	276.770	1197.100	231.200	4.381	66.539	39.013	20218.00	1370.090	341.100	674.000	506.080	306.900	1163.900	263.680	3.908	79.747	55.191	36884.75	2193.010	7150.42	77.30	27.08
JK52	37.175	300.980	995.900	302.400	292.700	1226.100	238.720	5.309	61.645	28.288	16498.48	1208.429	310.520	715.600	433.920	285.550	1181.900	241.600	3.928	73.843	49.161	34961.03	2070.070	6114.40	79.30	11.53
JK112	61.720	359.780	874.300	411.520	347.280	1201.200	289.120	5.203	71.912	39.219	27377.97	1818.262	374.190	593.300	630.720	337.370	1155.400	292.000	3.786	89.027	65.242	48557.95	2659.203	6627.52	79.60	18.66
JK53	48.968	314.310	950.400	330.720	301.690	1207.100	249.920	5.189	64.467	31.865	20475.28	1337.369	353.040	751.800	469.600	323.680	1164.000	278.080	4.418	76.819	49.058	28859.73	1849.983	6682.08	77.70	28.46
JK113	31.097	239.250	876.500	272.960	231.320	1226.300	188.640	4.141	58.567	32.551	10237.23	1028.666	265.480	652.000	407.200	246.540	1194.500	206.400	3.390	71.533	50.233	22641.97	1571.753	5926.24	79.40	36.34
JK103	118.693	462.940	1046.000	442.560	446.280	1205.400	370.240	7.101	74.575	30.146	34154.80	2118.191	491.160	717.000	636.800	449.120	1174.500	382.400	5.238	89.455	56.541	61009.36	3344.297	7564.22	77.80	14.91
JK95	44.969	308.890	886.800	348.320	295.040	1210.800	243.680	4.758	66.160	36.262	20351.32	1386.788	343.740	644.600	533.280	311.840	1165.000	267.680	3.834	81.862	57.772	38009.58	2293.232	7856.80	76.50	24.33
JK117	35.037	317.650	901.600	352.320	308.720	1206.700	255.840	6.659	34.820	21164.16	1476.011	346.230	668.300	518.080	323.770	1163.000	278.400	80.687	54.881	38735.57	2281.248	6816.48	78.60	20.49		
JK118	22.380	241.070	923.800	260.960	229.350	1228.300	186.720	5.726	30.544	12718.85	970.707	275.690	688.700	400.320	252.890	1194.700	211.680	70.927	48.688	24524.84	1526.613	5929.12	79.60	40.57		
JK119	60.558	385.750	847.400	455.200	372.680	1193.300	312.320	75.632	42.373	33027.04	2093.172	419.290	588.700	703.680	369.530	1155.900	319.680	94.036	69.466	64000.56	3249.136	5762.40	80.00	36.91		
JK120	31.073	272.890	836.100	326.400	236.070	1174.700	200.960	64.044	39.703	16831.04	1247.326	294.670	577.000	519.360	263.350	1164.800	226.080	80.787	60.708	32975.24	1989.495	6567.68	78.70	21.10		
JK121	28.146	302.380	844.100	358.240	289.440	1191.700	242.880	67.095	38.074	20299.47	1509.682	342.660	656.100	522.240	308.490	1179.300	261.600	81.010	57.230	36111.02	2227.887	7094.24	75.90	18.66		
JK20	41.178	274.460	908.600	302.080	259.170	1179.800	219.680	4.684	61.612	32.179	15080.50	1104.569	317.400	698.500	454.400	293.300	1141.400	256.960	4.099	75.566	49.811	29954.24	1820.794	9258.88	76.00	10.75
JK21	37.742	261.240	934.100	279.680	252.070	1199.900	210.080	4.728	59.284	29.574	13439.35	1035.391	280.560	629.200	445.920	252.850	1154.400	219.040	3.416	74.857	53.395	25692.40	1678.131	7564.80	80.80	24.31
JK51	32.813	312.050	924.300	337.600	300.760	1189.700	252.800	5.171	65.134	32.644	18849.75	1337.703	337.360	682.600	494.240	304.780	1143.400	266.560	4.030	78.809	53.489	36926.56	2038.928	5400.12	80.60	11.50
JK88	93.967	368.250	930.300	395.840	349.370	1139.000	306.720	5.899	70.529	33.465	25546.97	1659.594	408.840	709.200	576.480	354.760	1113.600	318.560	4.485	85.113	56.931	46181.96	2363.617	5795.84	77.80	20.66
JK44	29.120	273.060	895.400	304.960	263.920	1206.600	218.720	4.613	61.905	32.920	15153.30	1226.516	302.910	656.000	461.760	269.590	1176.900	229.120	3.518	76.175	54.069	37587.60	2075.428	7751.36	78.90	20.83
JK35	31.599	260.860	832.200	313.440	249.700	1205.100	207.200	4.173	62.766	36.538	15305.28	1197.480	286.520	660.100	434.080	265.290	1162.700	228.160	3.659	73.857	50.869	26759.50	1731.943	5432.91	80.30	21.41
JK79	48.962	304.440	918.300	331.520	295.730	1178.000	251.040	5.211	64.545	31.802	18286.09	1325.775	312.980	691.500	452.640	293.020	1154.700	253.760	4.047	75.419	49.992	29829.73	1831.459	4538.40	78.10	3