Bone Mineral Density, Body Composition and Dietary Intake in Asian Indian and Caucasian Males

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DEDICATION

I would like to dedicate this thesis to my family members who have believed in my ability to succeed in my master's degree. Without their support, motivation, encouragement and love, I would not have overcome the hindrances in my life throughout the previous year and a half. Also, I wish to dedicate this thesis to Dr. Deeptha Sukumar for the invaluable lessons and experiences she has offered and shaped me into who I am today. Dr. Deeptha Sukumar, your impact in my life will not be forgotten.

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ABBREVIATION AND DEFINITIONS OF TERMS

%BF	Body Fat Percentage
25OHD	25-hydroxy Vitamin D
$_{a}$BMD	Areal Bone Mineral Density
BMAD	Bone Mineral Apparent Density
BMI	Body Mass Index
BP	Blood Pressure
Ca	Calcium
DXA	Dual-Energy X-ray Absorptiometry
FFQ	Food Frequency Questionnaire
FM	Fat Mass
FN	Femoral Neck
HDL-C	High-Density Lipoprotein Cholesterol
ICMR	Indian Council of Medical Research
IDF	International Diabetes Federation
LDL-C	Low-Density Lipoprotein Cholesterol
LM	Lean Mass
LS	Lumbar Spine
MetS	Metabolic Syndrome
Mg	Magnesium
NCEP-ATP III	National Cholesterol Education Program - Adult Treatment Panel III
NIH	National Institute of Health
PTH	Parathyroid Hormone
RDA	Recommended Dietary Allowance
SAT	Subcutaneous Adipose Tissue
TG	Triglycerides
VAT	Visceral Adipose Tissue
WB	Whole Body

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ABSTRACT

Bone Mineral Density, Body Composition and Dietary Intake in Asian Indian and Caucasian Males

Abeer Ali Aljahdali

Background: The difference in osteoporotic fractures across different ethnic groups could be due to the differences in body composition and eating habits. The variation in areal bone mineral density ($_{a}$BMD), body composition and dietary intake between immigrant Asian Indians and Caucasians are unknown.

Objective: The objective of this study was to examine the differences in $_{a}$BMD, body composition and dietary intake between immigrant Asian Indians and Caucasians matched for age and body mass index (BMI). Also, we examined the relationship between body composition and $_{a}$BMD and how might the relationships differ between the two groups.

Design: A convenient sample of 32 healthy males who self-identified themselves as either Asian Indians (n=16) or Caucasians (n=16) were recruited for this cross-sectional study. Dual-energy x-ray absorptiometry (DXA) was used for measuring $_{a}$BMD and bone mineral content (BMC) at the lumbar spine, femoral neck, 33% radius of the non-dominant hand and whole body. Body composition was measured using whole body scan by DXA. Dietary intake was assessed using 24-hour food recall and food frequency questionnaires.

Results: There was no difference between groups in age and BMI. Lean mass was lower in Asian Indians compared Caucasians ($p=0.03$). $_{a}$BMD at measured sites were similar between two groups (all $p>0.05$). However, BMC was lower at all skeletal sites (all $p<0.05$). In all overall sample, lean mass correlated positively with $_{a}$BMD at the lumbar spine ($p<0.05$), but with $_{a}$BMD at the femoral neck in Caucasian males only ($p=0.03$). Fat mass did not correlate with $_{a}$BMD at any sites (all $p>0.05$). There were no differences in the correlation between body composition with $_{a}$BMD between Asian Indians and Caucasians. Regarding dietary intake, calcium and magnesium intakes were not different between the two ethnic groups (all $p<0.05$).

Conclusion: Asian Indian men have lower lean mass and BMC at different skeletal sites, but similar other body composition variables and dietary intakes compared to age and BMI matched Caucasians. Lean mass showed a positive correlation with $_{a}$BMD. Since the scope of this study was limited to examine the difference in bone density using $_{a}$BMD, the study highlights the need for further research to examine the variability in bone turnover between immigrant Asian Indians and Caucasians to see if the findings from biochemical properties and bone density are similar.

CHAPTER 1: INTRODUCTION

1.1 Introduction

Osteoporosis is a public health concern in the United States and around the globe. The burden of osteoporotic fracture entails financial, physical, and psychosocial aspects. Annually, approximately $10 to $15 billion is the financial cost that is directed to hospitals' care for subjects with osteoporotic fractures in the United States [1]. With increasing in life expectancy, the proportion of the elderly population is expected to increase. Aging is a risk factor for osteoporosis and hence osteoporotic fractures. During the last three decades, there has been an increase in hip fractures by 2 to 3 times in Asian countries. It has been estimated that more than half of the osteoporotic fractures will occur in Asian countries by 2050 [2].

Even though the prevalence of osteoporosis in men is lower than women, the expected increase in the men with osteoporosis is 89% compared to 69% in women by 2025 [3]. The mortality rate in men is higher due to osteoporosis and osteoporotic fractures compared to women in developed and developing countries such as India. In fact, Asian Indians have a lower areal bone mineral density ($_a$BMD) compared to Caucasians [4, 5,6].

Body composition differs between subjects from different ethnic groups. Asian Indians have higher body fat, visceral adipose tissue and subcutaneous adipose tissue but lower muscle mass compared to Caucasians. Due to the differences in body composition, Asian Indians have high prevalence of metabolic syndrome (MetS) and type 2 diabetes at young age and normal weight. Therefore, World Health Organization and International

Diabetes Federation have established specific cut-off values for BMI and waist circumference, respectively for Asians. Moreover, body composition has influences on $_{a}$BMD. Lean mass, for instance, has been shown to have a positive effect on bone. Higher body fat, visceral adipose tissue and subcutaneous adipose tissue might impact $_{a}$BMD negatively.

This thesis will focus on investigating the differences in $_{a}$BMD, body composition, and calcium and magnesium intake between healthy Asian Indian and age and body mass index-matched Caucasian males. Also, the study will examine the association between body composition and $_{a}$BMD in healthy young adults and within each ethnic group.

1.2 Aims and Hypotheses

1.2.1 Specific Aims and Hypotheses

Aim 1: To determine whether areal bone mineral density ($_{a}$BMD) at the lumbar spine, femoral neck, and 33% radius of the non-dominant hand and whole-body differ between Asian Indian and Caucasian men matched for age and body mass index (BMI)

- Hypothesis 1.A: Asian Indians will have lower whole-body $_{a}$ BMD compared to age, and BMI matched Caucasian men
- Hypothesis 1.B: Asian Indians will have lower $_{a}$BMD at the lumbar spine compared to age, and BMI matched Caucasian men
- Hypothesis 1.C: Asian Indians will have lower $_{a}$BMD at the femoral neck compared to age, and BMI matched Caucasian men
- Hypothesis 1.D: Asian Indians will have lower $_{a}$BMD at the 33% radius of the non-dominant hand compared to age, and BMI matched Caucasian men

Aim 2: To examine whether measured android fat, gynoid fat and lean mass differ between Asian Indian and Caucasian men matched for age and BMI

- Hypothesis 2.A: Android fat will be higher in Asian Indians compared to age, and BMI matched Caucasian men
- Hypothesis 2.B: Gynoid fat will be similar in Asian Indians compared to age, and BMI matched Caucasian men
- Hypothesis 2.C: Lean Mass will be lower in Asian Indians compared to age, and BMI matched Caucasian men

Aim 3: To determine whether dietary intake of calcium and magnesium differ between Asian Indian and Caucasian men matched for age and BMI.

- Hypothesis 3.A: Asian Indians will have lower intakes of dietary calcium compared to age, and BMI matched Caucasian men
- Hypothesis 3.B: Asian Indians will have similar intakes of dietary magnesium compared to age, and BMI matched Caucasian men

Aim 4: To determine the relationship between body composition and $_{a}$BMD at the lumbar spine and femoral neck in Asian Indian and Caucasian men matched for age and BMI.

- Hypothesis 4.A: Android fat will be negatively correlated with $_{a}$BMD at the lumbar spine, and femoral neck in Asian Indian and age and BMI matched Caucasian men

- Hypothesis 4.B: Gynoid fat will be positively correlated with $_{a}$BMD at the lumbar spine, and femoral neck in Asian Indian and age and BMI matched Caucasian men
- Hypothesis 4.C: Lean mass will be positively correlated with $_{a}$BMD at the lumbar spine, and femoral neck in Asian Indian and age and BMI matched Caucasian men
- Hypothesis 4.D: The correlation between android fat, gynoid fat and lean mass with $_{a}$BMD at the lumbar spine and femoral neck will differ for Asian Indian compared to age, and BMI matched Caucasian men

1.2.2 Exploratory Aims and Hypotheses

Exploratory Aim 1: To determine whether the percentage of Asian Indians who meet the calcium recommended dietary allowance (RDA) by the Indian Council of Medical Research will differ from the percentage of Caucasian men who meet the calcium RDA by the National Institute of Health

- Hypothesis: the percentage of Asian Indians who meet the calcium RDA by the Indian Council of Medical Research will be lower than the percentage of Caucasian men who meet the calcium RDA by the Institute of Medicine

Exploratory Aim 2: To examine whether measured visceral adipose tissue and subcutaneous adipose tissue differ between Asian Indian and Caucasian men matched for age and BMI.

- Hypothesis 2.A: Visceral adipose tissue will be higher in Asian Indian compared to age, and BMI matched Caucasian men
- Hypothesis 2.B: Subcutaneous adipose tissue will be higher in Asian Indian compared to age, and BMI matched Caucasian men

Exploratory Aim 3: To determine whether bone mineral apparent density (BMAD) at the lumbar spine, femoral neck, and 33% radius of the non-dominant hand and whole-body differ between Asian Indian and Caucasian men matched for age and BMI

- Hypothesis 1.A: Asian Indian men will have similar whole-body BMAD compared to age, and BMI matched Caucasian men.
- Hypothesis 1.B: Asian Indian men will have similar BMAD at the lumbar spine compared to age, and BMI matched Caucasian men.
- Hypothesis 1.C: Asian Indian men will have similar BMAD at the femoral neck compared to age, and BMI matched Caucasian men.
- Hypothesis 1.D: Asian Indian men will have similar BMAD at the 33% radius of the non-dominant hand compared to age, and BMI matched Caucasian men.

CHAPTER 2: LITERATURE REVIEW

2.1. Definition of Asian Indian and Caucasian Ethnicity

Asian Indians are natives of first or subsequent generation immigrants who can trace their ancestry to any country of the Indian subcontinent. These countries are India, Bangladesh, Nepal, Pakistan and Sri Lanka. Caucasians are those who trace their ancestry to non-Hispanic ethnicity and who do not belong to any other ethnic group.

2.2. Osteoporosis

2.2.1 Definition of Osteoporosis

Osteoporosis is defined by the National Institutes of Health "as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture." [1] Osteoporosis has metabolic consequences which go beyond the skeletal system; it impacts the body homeostasis [7]. Bone strength contains two major components, which are bone density and bone quality. Areal bone mineral density ($_a$BMD) is expressed as the density of the bone in gram (gm) per centimeter squared (cm^2), while bone quality composes of various factors about bone structure, bone turnover, "damage accumulation (e.g., microfractures) and mineralization." Multiple assessment tools have been developed to measure some aspects of bone quality, but most of them still applied in a research setting, not in clinical applications [7]. Since bone density accounts for 70% of bone strength, bone mineral density is used as a surrogate measure of bone strength [1]. Therefore, the diagnosis of osteoporosis is based on $_a$BMD measured by Dual Energy X-ray Absorptiometry (DXA) [8].

2.2.2 Diagnosis of Osteoporosis

According to the World Health Organization (WHO), osteoporosis is diagnosed if a subject's T score is ≥ 2.5 standard deviations (SD) less than the reference $_{a}$BMD measured for healthy young White women between the age 20 to 29 years old [9]. The T-score is calculated by taking the difference between a subject's $_{a}$BMD value at a particular site and average $_{a}$BMD of young adults matched the subject in their sex and ethnicity at the same skeletal site and dividing the difference by "young adult population SD" as follow, T score= $\frac{\text{Subject's aBMD} - \text{Average young adults aBMD}}{\text{Young adult population SD}}$ [10].

2.2.3 The Common Sites to Measure Areal Bone Mineral Density

The bone is made of two structural units, which are cortical bone and trabecular bone. The cortical bone, or what is called compact bone, is the hard-out layer of the bone. It covers the internal part of the bone. On the other hand, trabecular bone is a spongy structure which resembles the structure of honeycomb. The distribution of the two bone structures varies across the skeletal system. For example, vertebrae, hips, forearm and proximal humerus have a higher percentage of trabecular bone than cortical bone. As a result, the previously mentioned sites are common sites for osteoporotic fractures, and they are commonly used to measure $_{a}$BMD and assess the risk of osteoporosis [11].

2.2.4 Normal Development of the Bone

As a normal development of bone, the first two decades of an individual's life is where the accumulation of bone mass occurs. During the puberty, the calcium intake is used to build the skeleton. Also, calcium is stored in the skeleton to be used in maintaining calcium homeostasis during periods of calcium deficiency or insufficiency.

In addition to calcium, vitamin D intake and exercise help in achieving optimal peak bone mass (PBM) during puberty [12]. PBM accounts for approximately 60 % of bone mass in adulthood [7]. Therefore, PBM is an essential factor in the susceptibility to fractures in later life. From the third decay until the end of the fourth decade, this stage is considered as a maintenance stage, where the bone formation equals bone resorption [13]. After that, the bone loss starts with a rate differs between men and women. The rate is higher in women during menopause due to the decline in estrogen level **Figure 1**[14]. Ultimately, the preventive approach of osteoporosis involves two significant dimensions. The former strategy is achieving optimal PBM during childhood and adolescence periods, while the latter approach is to slow the rate of the inevitable bone loss which is a standard physiological change with aging[1].

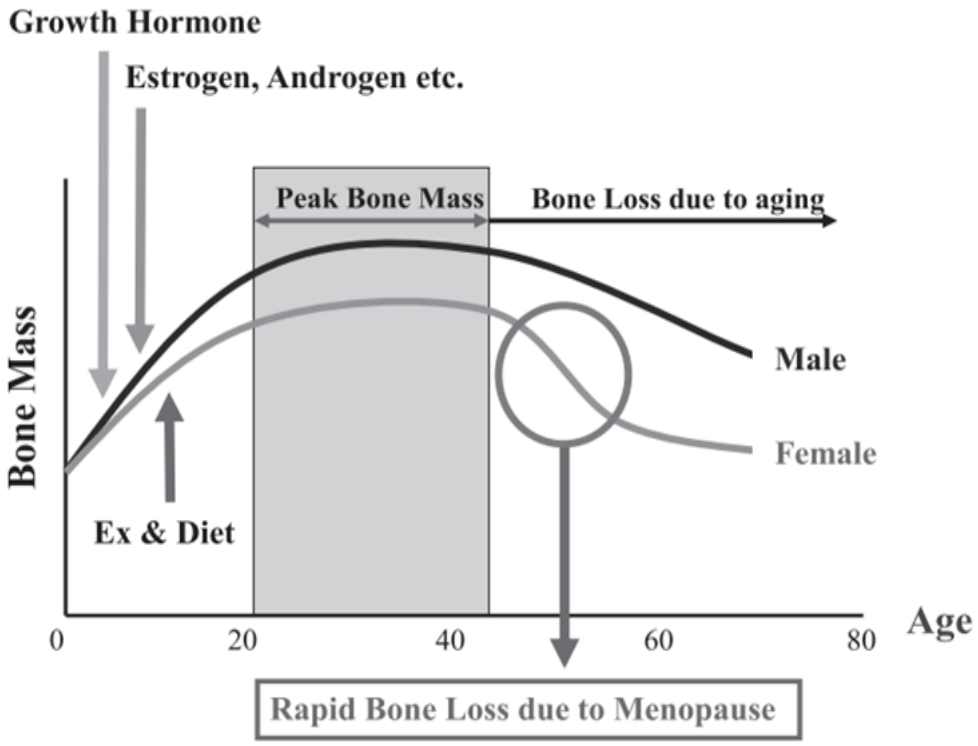

Figure 1: The Normal Development of Bone Across Lifespan. Adapted from Ishimi, 2015 [14]

2.2.5 The Prevalence and Burden of Osteoporosis

In 2005, the incidence of fractures was more than 2 million in the United States[15]. The prevalence of osteoporosis in developing countries is not well established because of the scarcity of the studies conducted on those populations [11]. In India, for example, there is no an official registry of fractures at the national level, but some hospitals have their own fractures registries [16]. It was estimated that 26 million of Asian Indians had osteoporosis in 2003 and 36 million in 2013 [17]. In 2015, 10% of the total deaths happened in India was due to fractures according to the cause of death certificate. [18].

The burden of osteoporotic fracture entails financial, physical and psychosocial aspects. Annually, approximately $10 to $15 billion is the financial cost that is directed to hospitals' care for subjects with osteoporotic fractures in the United States [1]. In India, the total cost of a hip fracture surgery had been estimated to be 772 US dollars in public hospitals with an average hospital stay of 15 days, while the cost was 2360 to 3860 US dollar in private hospitals with an average hospital stay of 5 to 6 days [16].

There is an increase in life expectancy and the elderly population around the world. In 1947, the average life expectancy at birth in India was 31 years, while the average life expectancy now is 67.14 years[19]. The percentage of elderly residents in Asian countries will be 9.3% in 2025. As a matter of fact, aging increases the risk of osteoporosis and fall. During the last three decades, there has been an increase in the hip fractures by 2 to 3 times in Asian countries. It has been estimated that there will be more than half of the osteoporotic fractures will occur in Asian countries by 2050 [2].

2.2.6 Osteoporosis in Men

It is a well-established fact that men have higher $_{a}$BMD than women at any skeletal sites and any stage of life. However, the incidence of hip fractures is higher in Asian Indian men compared to women counterparts. The incidence of hip fracture was 32 cases per 100,000 (95% CI 16 – 57) in Asian Indian men compared to 14 cases per 100,000 (95% CI 4 – 35) in women between the 40 to 44 years of age [20]. Even though the incidence rates did not statistically differ between sexes, it provides a clue for the high susceptibility of hip fractures in Asian Indian men. Moreover, the mortality rate is higher in men due to osteoporotic fractures compared women [21]. The age-standardized mortality

ratio (SMRs) were 3.17 vs. 2.18 for the proximal femur, 2.38 vs. 1.66 for the vertebral fractures, and 1.45 vs. 0.75 for minor fractures in men and women, respectively[21]. In 2015, the total number of deaths due to fractures among Indian men was 5200, while only 1872 was the number of deaths in Indian women due to fractures [18].

Due to the economic prosperity, the life expectancy is now higher in both men and women. With an increase in life expectancy, the osteoporosis and osteoporotic fractures will be more prevalent in older men because aging is one of the predisposing factors in developing fractures. In 2025, the number of hip fractures in men will be 800,000 cases per a year compared to 424,000 cases in 2000. In women, the number of cases was more than one million in 2000, and it will be 1.8 million cases in 2025. Even though the number of cases is higher in women than men, the percentage of increase in cases with hip fracture will be 89% in men compared to 69% in women between 2000 to 2025 [3]. Ultimately, it is vital to address the problem of osteoporosis in men like the attention has been given to osteoporosis in women.

2.2.7 Areal Bone Mineral Density in Asian Indians versus Caucasians

The difference in $_{a}$BMD between Caucasians and Asian Indians has been investigated in several studies, and the findings are intriguing. Some researchers reported that $_{a}$BMD is lower in Asian Indians compared to Caucasians. The $_{a}$BMD of 808 healthy Asian Indians between the age of 20 to 29 and BMI 18.5 to 25 kg/m^2 was measured at the lumbar spine (L_1-L_4), non-dominant forearm, femoral neck and whole body. By comparing the Asian Indian's $_{a}$BMD with the data collected from the Third National Health and Nutrition Examination Survey (NHANES III), Asian Indians have

significantly lower $_a$BMD at all sites compared to Caucasians (p-value < 0.001)[4]. Another study conducted to quantify the percentage of difference in $_a$BMD between Asian Indian males and Caucasians. Asian Indians had $_a$BMD at the femoral neck, total hip and lumbar spine (L_1-L_4) that were lower than Caucasians' $_a$BMD by 15.2%, 17.9%, and 16.5%, respectively [6]. Makker and colleagues calculated the prevalence of osteoporosis and osteopenia in 1004 healthy Asian Indian adults twice using normative $_a$BMD values been collected in the study, and data for Caucasians provided by DXA's manufacture. They stated that the use of Caucasians' $_a$BMD as a reference standard had a "limited validity" in Asian Indians [5]. On the other hand, a site-specific difference in $_a$BMD was observed in healthy and physically active Asian Indian men. Compared to Caucasians, Asian Indians had similar $_a$BMD at the femur trochanter, and higher $_a$BMD at femur neck and total femur but lower $_a$BMD at the lumbar spine. The authors, however, stated that the generalizability of study findings was limited because of the sample studied composed of paramilitary personnel who were taller, physically active and consumed more calcium and protein than general population [22].

The difference in skeletal size between Asian Indians and Caucasians has been considered as one of the possible causes for lower $_a$BMD in Asian Indians compared to Caucasians. Therefore, adjusting for the frame size in comparing $_a$BMD between Asian Indians and Caucasians was used before. For that, Roy et al. conducted a study to investigate the difference in $_a$BMD and volumetric bone density between Asian Indian and European women with and without adjusting for the skeletal size. They found that Asian Indians had lower $_a$BMD at the lumbar spine (L_1-L_4), total hip and the whole body

compared to European women ($p < 0.05$). The differences in $_a$BMD at the lumbar spine, however, was eliminated by calculating bone mineral apparent density (BMAD) (gm/cm^3) at the lumbar spine, using the following equation $\frac{Areal\ Bone\ Mineral\ Density\ at\ \ Lumbar\ Spine(gm/cm^2)}{Height\ (m)}$. The BMAD is a way to adjust for the skeletal size. The lack of difference in bone density between the two groups was supported by the results of volumetric bone density (mg/cm^3) measured by peripheral quantitative computed tomography at trabecular and total BMD (mg/cm^3) at the distal radius [23]. Similarly, the differences in $_a$BMD disappeared at femoral neck and total hip when $_a$BMD was adjusted for weight and height between: 1) Caucasians and US Asian men [24]; 2) Caucasians and Asian Indian men [25], 3) Europeans and Asian Indian men [26]; 4) Europeans and Asians Indian women [27]; and 5) Caucasians and Asian Indian women [28]. Cundy and colleagues stated that Asian Indians has lower $_a$BMD because of their short stature compared to Europeans and Caucasians [27]. Asian Indians might have higher bone density than Caucasians when the $_a$BMD measurements adjusted for the skeletal size. For instance, Mehta et al. found that Asian Indian women had a higher BMAD (gm/cm^3) at femoral neck compared to Caucasians matched for age [28].

DXA calculates $_a$BMD at any site using the following formula $\frac{Bone\ Mineral\ Content\ (gm)}{Projected\ Area\ (cm^2)}$. The denominator in the $_a$BMD equation is only influenced by a two-dimensional shape of the bone. In fact, the projected area for whole body $_a$BMD is influenced by subject's height and width. By doing so, bone' depth or how long the DXA's "beam has to pass" through the bone is ignored. Bone depth is influenced by the size of bone. However, the volume or the bone size is not accounted for in $_a$BMD. Carter

et al. stated that taller subjects will have higher $_{a}$BMD compared to shorter subjects eventhough they both have simlar volumetric bone denisty [29]. Ignoring the impact of skeletal size in comparing $_{a}$BMD between different ethnic groups particularly when they differ in their skeletal dimensions is the reason for the variation in $_{a}$BMD [27,30,31].

2.3 Metabolic Syndrome

2.3.1 Definition of Metabolic Syndrome

Metabolic syndrome (MetS) is a cluster of proatherogenic hormonal, clinical, and anthropometric abnormalities that predispose an individual to develop type 2 diabetes and cardiovascular diseases. These risk factors are 1) impaired glucose metabolism or insulin insensitivity; 2) obesity or central obesity; 3) high blood pressure (BP); 4) dyslipidemia characterized by low high-density lipoprotein cholesterol (HDL-C), high low-density lipoprotein cholesterol (LDL-C).

Although there are multiple diagnostic criteria for MetS developed by many organizations, there are two diagnostic criteria are widely used in research, which are the National Cholesterol Education Program's Adult Treatment Panel III report (NCEP/ATP III) and the International Diabetes Federation (IDF) criteria. The NCEP/ATP III was established by American Heart Association (AHA) and National Heart Lung and Blood Institute. According to NCEP/ATP III, a subject is diagnosed with MetS if s/he has three of the following risk factors 1) abdominal obesity (waist circumference for men and women > 102, and > 88 cm, respectively); 2) elevated serum Triglycerides [(TG) ≥ 150 milligrams per deciliter (mg/dL)] ; 3) low serum HDL-C (men < 40 and women < 50 mg/dl) ; 4) BP ≥ $\frac{130}{85}$ millimeters of mercury (mmHg); or 5)elevated fasting plasma

glucose [(FBG) ≥ 100 mg/dl]. The ethnic difference in the body fat percentage has considered in the diagnostic criteria by the IDF. According to IDF criteria, a subject is diagnosed with MetS if s/he has central obesity proved either by the waist circumference **Table 1** or BMI > 30 kg/m^2. In addition to central obesity, the subject has to have two of the following risk factors: 1) elevated TG ≥ 150 mg/dL or treatment for this lipid abnormality; 2) low HDL-C (men < 40 and women < 50 mg/dl) or treatment for this lipid abnormality; 3) BP ≥ $\frac{130}{85}$ (mmHg) or treatment of previously diagnosed hypertension; 4) FPG ≥ 100 mg/dL or previously diagnosed type 2 diabetes [32].

TABLE 1
Waist Circumference cut-off for Different Ethnic Groups [32]

Country/Ethnic group		Waist circumference
Europids In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes	Male	≥ 94 cm
	Female	≥ 80 cm
South Asians Based on a Chinese, Malay and Asian-Indian population	Male	≥ 90 cm
	Female	≥ 80 cm
Japanese	Male	≥ 90 cm
	Female	≥ 80 cm
Ethnic South and Central Americans Use South Asian recommendations until more specific data are available	Male	≥ 90 cm
	Female	≥ 80 cm
Sub-Saharan Africans Use European data until more specific data are available	Male	≥ 94 cm
	Female	≥ 80 cm
Eastern Mediterranean and Middle East (Arab) populations Use European data until more specific data are available	Male	≥ 94 cm
	Female	≥ 80 cm

Adapted from International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome, 2006.

2.3.2 Metabolic Syndrome in Asian Indians

MetS is very prevalent among Asian Indians in the U.S [33, 34, 35] as well as in India [36]. The age-adjusted prevalence of MetS in adults age between 19 to 81 living in the U.S was 32.7%, and 38.2%, using NCEP/ATP III, and IDF diagnostic criteria, respectively[33]. Flowers and colleagues reported a slightly lower prevalence of MetS (27%) in their sample using IDF diagnostic criteria [34]. On the other hand, the prevalence of MetS in

Caucasian adults between the age 38 to 97 was 23.5% and 18.2% in men and women, respectively [37]. It has been proved that MetS occurs in Asian Indians at younger ages compared to Caucasians[38].

Asians Indians have higher FM and %BF but lower muscle mass compared to Caucasians and other Asians. The regional fat distribution is also reported to be higher in Asian Indians compared to Caucasians. Raji et al. observed that healthy Asian Indian adults with normal weight had higher total abdominal fat and visceral adipose tissue (all p= 0.04) compared to age, sex, and BMI matched Caucasians. Subcutaneous adipose tissue did not differ between the groups [39]. On the other hand, Chandalia and colleagues found no significant difference in subcutaneous adipose tissue as well as visceral adipose tissue between Asian Indian and Caucasian men [40]. They investigated the difference in the size of adipose cells in subcutaneous adipose tissue. Asian Indians had higher cell size on average compared to Caucasians (p-value=0.0001) [40]. Some researchers have linked the size of adipose cells positively with type 2 diabetes and insulin resistance[38]. Misra et al. proposed to lower waist circumference cut-off points for Asian Indians further. The suggested cut-off values are 78 cm and 72 cm in Asian Indian men and women, respectively to identify abdominal obesity [41].

Interestingly, environmental and lifestyle factors come to play in predisposing Asian Indians to obesity and MetS. Like the nutrition transition seen in western countries, Asian Indians are affected by this transition as well due to the economic growth. More packaged and processed food are available in supermarkets and food outlets than before. Hence the intake of saturated fat, cholesterol, trans fatty acids , refined carbohydrates and

calories increases in Asian Indians' diet[38]. The daily energy intake in Asian Indian adults exceeded their basal metabolic rate[33], which might predispose them to weight gain. Nonetheless, fruit and vegetable and fiber intakes are low. Shah et al. have measured the fiber intake in the diet of young Asian Indian adults. The average fiber intake was 20 ±10 gm and 18± 8 gm/day in men and women, respectively[33], which is lower than the recommendation of 38 gm/ day for men and 25 gm/ day for women[42]. In addition to the eating pattern, the physical inactivity is very prevalent among Asian Indians compared Caucasians[25,38]. There has been a consensus to emphasis more on the physical activity recommendations to Asian Indians to curb the obesity and MetS. The recommendation is to have 60 minutes of physical activity every day including both resistance and aerobic exercise [43]. All these factors make MetS is very common among Asian Indians.

2.3.3 Metabolic Syndrome and Osteoporosis

The relationship between MetS and osteoporosis is attributed to multiple factors. Most importantly, osteoporosis and MetS are both caused by genetic, behavioral and lifestyle factors and the interaction between these factors. For example, aging is a predisposing factor to bone loss and osteoporosis [44]. At the same time, aging leads to MteS directly or indirectly via an increase in %BF, waist circumference and blood pressure, which are predisposing factors to MetS [45]. The components of MetS have been associated with either increase or decrease the incidence osteoporosis [46,47]. Obesity or central adiposity, which is the hallmark of MetS, associated with heavier weight and higher mechanical load on bone. The bone mass and density increase in response to the higher load [44,46,48]. It has been estimated the FM only contribute up to 16%[43] - 27%[44] of

body weight in White men. As a result, fat mass is unlikely to explain the positive effect alone. However, the FM contributes to a large percentage of body weight in the Asian population, which could be the reason for the inconsistency in the findings of the effect of FM on $_{a}$BMD. Moreover, FM has been recognized recently as an active endocrine organ which secretes active metabolites, and hormones involved in the bone metabolism whether favorably such as 17-estradiol or negatively such as inflammatory cytokines [44,46]. Furthermore, osteoblast and adipocyte cells are originated from the same progenitor in the bone marrow, which is the mesenchymal stem cell [44,46,48]. The differentiation of mesenchymal stem to either cell is influenced by some cytokines secreted by adipocytes[49].

2.3.3 Previous Findings on the Relationship Between Metabolic Syndrome and Osteoporosis

Muhlen et al. investigated the association between MetS and osteoporosis in Caucasian adults. They found that the age-adjusted $_{a}$BMD at the femoral neck and lumbar spine were higher in participants with MetS than participant without MetS ($p \leq 0.05$). However, the protective effect of MetS on $_{a}$BMD reversed by adjusting for BMI in addition to age but was not significant at any skeletal site. On the contrary, Yaturu and colleagues reported that individuals with MetS had lower $_{a}$BMD at femoral neck compared to the control group (0.892 ± 0.009 vs 0.958 ± 0.08 gm/cm^2, $p < 0.01$)[50]. Some researchers observed a sex-dependent association between MetS and $_{a}$BMD. Positive associations between $_{a}$BMD at the lumbar spine and total hip in women with MetS but not in men[45,51]. Similarly, the incidence of fracture in subjects with MetS and without

MetS differs between men and women. It has observed that women with MetS were 276% more likely to develop non-vertebral osteoporotic fractures compared women without MetS during two years of follow up (Odds Ratios (OR) = 3.76, 95% CI 1.27–11.13, p = 0.02). The incidence of osteoporotic fractures, however, was not significant for men with MetS compared to control (OR = 2.48, 95% CI 0.49 – 12.60, p = 0.27)[37].

The association between osteoporosis and every component of MtS has been investigated. For example, the central obesity or waist circumference has been found to negatively associated with $_a$BMD at the lumbar spine, femoral neck and total hip (all $p < 0.05$) in men with MetS but not in women[51]. Along the same line, other studies confirmed the negative association in young and older Korean adults (p-value < 0.05)[45] and Dutchmen (p-value = 0.004)[52].

2.4 Bone Regulating Nutrients

Lifestyle behaviors influence 20% to 40% of the PBM attainment. One of these lifestyle behaviors is nutrition. In fact, nutrition plays a crucial role in the bone mineral acquisition, maintenance of bone, and protection against the regular load imposed on bone [53]. Many nutrients are essential for the bone, but calcium, vitamin D, protein and magnesium are the most powerful nutrients.

2.4.1 Calcium

Calcium is widely distributed in the body and in average, a subject has one kilogram of calcium in his/her body[54]. About 99% of body calcium is in the skeletal system and teeth[54], and only 1% is in the extracellular fluid [55]. Bone is composed of three components: 1) mineral, which accounts for 60%, 2) an organic matrix, which accounts

for 30%, and 3) water, which accounts for 10% of total bone. The mineral component contains calcium and phosphate, and both compose of what is called Hydroxylapatite (Ca 10 $[PO_4]_6$ $[OH]_2$). Calcium, also, is a crucial element in contributing to the hardness of cortical bone and hence bone strength by increasing the bone mass[54,56]. Therefore, calcium that is stored in the skeleton works as a calcium reservoir to replenish calcium during periods of low calcium intake or impaired absorption. In order for stored calcium to maintain normal calcium homeostasis, the bone resorption process must start.

Among one of the modifiable factor for good bone mass during the PBM in childhood and adolescence and bone maintenance in adulthood is calcium intake [54]. Calcium contributes to the bone health through two major ways. The former approach is during the PBM since calcium serves as a structural unit in bone tissue. The latter role is during the adulthood. Adequate intake of calcium prevents bone resorption and might slow the rate of bone loss. On the other hand, low serum calcium concentration triggers the release of the Parathyroid hormone (PTH) to initiate the bone resorption process. The purpose of bone resorption is to free some calcium from the skeleton to maintain calcium homeostasis. In addition to its direct role as a component in the Hydroxylapatite, calcium triggers the osteoclast and osteoblast differently for the sake of bone formation. A higher serum concentration of calcium in the extracellular fluid will be detected by calcium-sensing receptor (CaSR) to promotes the proliferation and differentiation of the osteoblast and hereafter bone formation. While osteoblast flourishes, osteoclast undergoes apoptosis [56].

According to the recommended dietary allowance (RDA) established by Food and Nutrition Board, Institute of Medicine, healthy Caucasian adults- males and females- between the ages of 20 to 50, the RDA for calcium is 1000 mg per day **Table 2** [57]. For Asian Indians, on the other hand, the RDA for calcium is lower for the same age group. Healthy Asian Indian males and females are required to consume 600 mg per day to meet the RDA established by Indian Council of Medical Research (ICMR) in 2009 **Table 3** [58]. The ICMR recommendation in 2009 is higher than the calcium RDA recommendation in 2000, which was 400 mg per day. There has been some suggestion to revise and increases the RDA for Asian Indians given the high prevalence of vitamin D defsiceiy[59]. The ICMR committee itself express its willingness to update the RDA once sufficient research studies are conducted to establish the new recommendation[58]. Dairy products are a good source of calcium, but other food items are rich in calcium as well **Table 4** [57]

TABLE 2
Calcium Requirement According to Institute of Medicine. [57]

Age group	Calcium requirement (milligram [mg] per day)
Male	
19 - 30 years	1,000
31 - 50 years	1,000

Adapted from National Institutes of Health. Website. Available from https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/

TABLE 3
Calcium Requirement According to Indian Council of Medical Research[58]

Age group	Calcium requirement (milligram [mg] per day)
Men (≥ 19 years)	600

Adapted from to Indian Council of Medical Research. Website. Available from http://icmr.nic.in/final/RDA-2010.pdf

TABLE 4
Food Sources of Calcium[57]

Food items	Calcium, (in milligram [mg]) per serving
Yogurt, plain, low fat, 8 ounces	415
Mozzarella, part skim, 1.5 ounces	333
Sardines, canned in oil, with bones, 3 ounces	325
Yogurt, fruit, low fat, 8 ounces	313–384
Cheddar cheese, 1.5 ounces	307
Milk, nonfat, 8 ounces	299
Soymilk, calcium-fortified, 8 ounces	299
Milk, reduced-fat (2% milk fat), 8 ounces	293
Milk, buttermilk, low-fat, 8 ounces	284
Milk, whole (3.25% milk fat), 8 ounces	276
Tofu, firm, made with calcium sulfate, ½ cup	253
Salmon, pink, canned, solids with bone, 3 ounces	181
Cottage cheese, 1% milk fat, 1 cup	138
Frozen yogurt, vanilla, soft serve, ½ cup	103
Turnip greens, fresh, boiled, ½ cup	99
Kale, fresh, cooked, 1 cup	94
Ice cream, vanilla, ½ cup	84
Chinese cabbage, bok choy, raw, shredded, 1 cup	74
Bread, white, one slice	73
Pudding, chocolate, ready to eat, refrigerated, 4 ounces	55

Adapted from National Institutes of Health. Website. Available from https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/

2.4.2 Vitamin D

Vitamin D is a fat-soluble vitamin and a steroid hormone. Sun exposure is a significant source of vitamin D. During the exposure to sunlight particularly ultraviolet B radiation, a vitamin D precursor in the skin, 7-dehydrocholesterol, is converted to previtamin D. Then, previtamin D transforms to vitamin D_3. For achieving its biological

function, vitamin D_3 undergoes two hydroxylation steps. One step is in the liver by 25-hydroxylase and the second one is in the kidney by 1-alpha-hydroxylase. Also, fatty fish and fortified food and supplementations are the two other sources of vitamin D[60].

Vitamin D has important roles in calcium and phosphorus absorption in the small intestine and maintenance of calcium homeostasis. The proportion of dietary calcium absorbed increases from 10 - 15% to 30- 40% in the presence of adequate vitamin D. Similarly, the percentage of phosphorus absorbed is 80% in the presence of vitamin D but 60% in case of vitamin D deficiency [60]. Vitamin D enhances the absorption of calcium and phosphorus by opening the calcium channels in the small intestine and simulating the synthesis of the calcium binding protein. The calcium binding protein makes the environment favorable to absorb calcium and phosphorus and promotes bone mineralization. In addition, vitamin D contributes to the maintenance of the of bone turnover rate, and hence the incidence of fractures[61]. Osteopenia and osteoporosis could be caused or exacerbated by vitamin D deficiency[61] **Figure 2**.

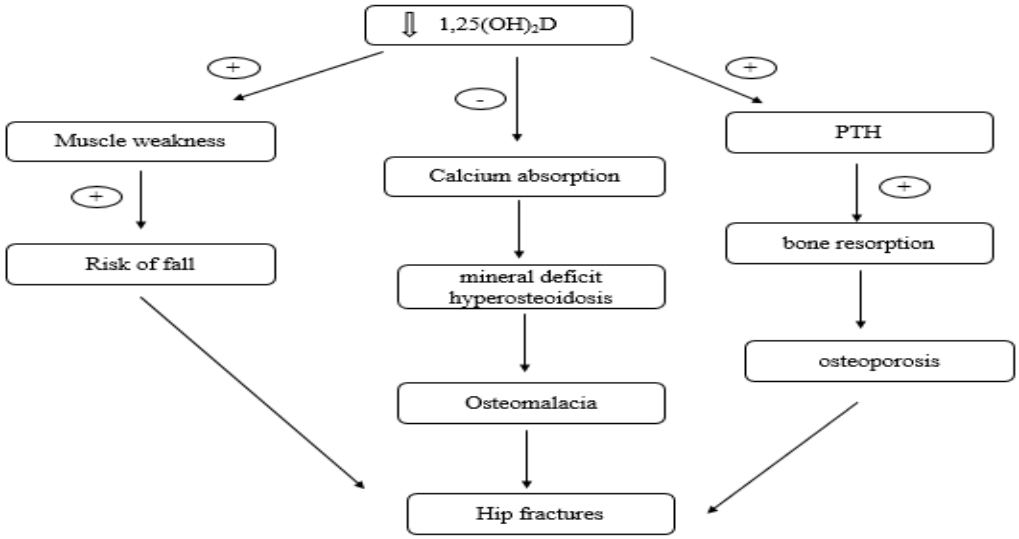

Figure 2: The Physiological Mechanisms of Osteopenia, Osteoporosis, and Fractures due to Vitamin D Deficiency. Adapted from Lips *et al*., 2011 [61]

2.4.3 Protein

Protein is a structural component of the bone; it contributes to the bone mass and bone volume by 33% and 50%, respectively [62]. Adequate intake of protein is essential to compensate the protein that lost during the proteolysis in bone remodeling process in bone matrix. The interaction between dietary protein and bone could be summarized into four major roles. Firstly, adequate intake of protein facilitates calcium absorption in the small intestine. Calcium absorption is facilitated by the intake of protein or amino acid [63]. With low protein intake of < 0.8 gm/kg/day, the calcium absorption will be low. As a result, the PTH will be activated to start the bone resorption and release calcium from

bone[63]. Secondly, protein provides a structural unit to the bone matrix. Thirdly, adequate intake of protein enhances the concentration of Insulin-like Growth Factor 1 (IGF-1). Larsson et al. found a positive association between protein intake and the serum concentration of IGF-1[64]. Finally, adequate protein intake maintains the muscle mass which works as a supportive and resistance against the fractures. Inadequate protein intake impacts the bone mass, strength and structure negatively even if adequate intake of calcium, vitamin D and other macronutrients are met [53]. A diet high in protein has a positive impact on the bone mass and lowering fractures risk if the diet coupled with adequate intake of calcium[62].

2.4.4 Magnesium:

Magnesium is one of the most abundant elements in the body. Specifically, magnesium takes rank 4 in term of its abundance in the body as a cation, and it makes a large contribution to the intracellular fluid in the body. Bone holds approximately 50% to 60% of total body magnesium [65,66]. Of that quantity, about one-third of bone magnesium is located in the cortical bone. Specifically, on the hydroxyapatite 's surface or "in the hydration shell around the crystal" to be a reservoir of magnesium when it is needed[15]. The small intestine and kidney are the primary sites for magnesium absorption and excretion, respectively [67]. Magnesium is a very active element in the body. To illustrate, it has a role in more than 300 metabolic reactions in the body. To name a view, it has a role in the protein, DNA, and RNA synthesis, production of the energy, reproduction, and others [68].

Magnesium is beneficial to bone mineral density. Higher intake of magnesium- both from dietary sources and supplements- was associated with higher the whole body $_{a}$BMD in White women and men (all p-value = 0.002). Ryder and colleagues concluded that there was an increase of 2% in the whole body $_{a}$BMD with every increase in magnesium intake by 100-mg per day[69]. Dietary magnesium intake correlated positively with the $_{a}$BMD at the trochanter (p-value < 0.05) in women and with the $_{a}$BMD at the radius (p-value < 0.01) and trochanter (p-value < 0.05) in men [70]. New et al. reported that urinary pyridinoline and urinary deoxypyridinoline, markers of bone resorption, were correlated negatively with magnesium intake (all p-value < 0.005). Moreover, magnesium intake accounted for 12.3% in the variation of pyridinoline excretion and 12.1% in the variation of deoxypyridinoline excretion [71].

In a human culture of osteoblast, magnesium showed an enhancement of bone formation [72]. As a structural component, lack of magnesium in bone formation impacts the size of hydroxyapatite crystals negatively by increasing the size of the crystals and makes the crystals well organized. Because of the better organization of the large crystals, the newly formed bone is fragile[65] and less capable of bearing load. Adequate presence of magnesium strengthens the bone by making the crystal small and not arrange correctly. Additionally, magnesium deficiency hinders the bone formation process by reducing the number and activity of osteoblast, while increasing the number of osteoclast[65]. Magnesium exerts another beneficial role in bone indirectly via the participation in calcium hemostasis. Hypomagnesemia decreases the release of PTH to the circulation when it is needed. In fact, PTH is the major stimulating factor to the

conversion of 25 (OH)D to the active form of vitamin D, which is 1.25 (OH_2) D, in the kidney. With the lack of PTH and 1.25 (OH_2) D, calcium absorption and bone mineralization[65,73].

Magnesium could be found in many animals and plant food items such as green leafy vegetables, whole grains, and nuts and seeds **Table 5** [74]. Interestingly, the increased intake of magnesium will increase the intake of other nutrients important to bone as well such as calcium and phosphorus. There were positive correlations between the consumption of dietary magnesium and the intake of other nutrients such as calcium ($r = 0.498$, p-value < 0.001)[75], ($r = 0.64$, p-value < 0.001)[69] and ($r = 0.319$, p-value <0.001)[76], phosphorus ($r = 0.58$, p-value < 0.001)[75], and ($r = 0.285$, p-value <0.001)[76], potassium ($r = 0.84$, p-value < 0.001), and protein ($r = 0.73$, $P < 0.001$)[69].

For a healthy Caucasian between the ages 30 to 70, the RDA for magnesium ranges from 310 to 420 mg per day depending on the age and sex **Table 6**[77]. For healthy Asian Indian male and females, RDA for magnesium is 340 mg and 310 mg, respectively **Table 7**[58].

TABLE 5
Food Sources of Magnesium[74]

Food items	Magnesium, (in milligram [mg]) per serving
Rice bran, crude [118 gram (1.0 cup)]	922
Molasses [337 gram (1.0 cup)]	816
Seeds, pumpkin and squash seed kernels, dried [129 gram (1.0 cup)]	764
Mothbeans, mature seeds, raw [129 gram (1.0 cup)]	764
Soybeans, mature seeds, raw [186 gram (1.0 cup)]	521

Seeds, sesame seeds, whole, dried [144 gram (1.0 cup)]	505
Nuts, almonds, oil roasted, without salt added [157 gram (1.0 cup whole kernels)]	430
Cocoa, dry powder, unsweetened, processed with alkali [86 gram (1.0 cup)]	409
Nuts, cashew nuts, dry roasted, without salt added [137 gram (1.0 cup halves and whole)]	356
Oats [156 gram (1.0 cup)]	276
Peanuts, all types, dry-roasted, without salt [146 gram (1.0 cup)]	260
Bulgur, dry [140 gram (1.0 cup)]	230
Seeds, hemp seed, hulled [30 gram (3.0 tablespoons)]	210
Nuts, walnuts, English [117 gram (1.0 cup, chopped)]	185
Spinach, cooked, boiled, drained, without salt [180 gram (1.0 cup)]	157
Chard, swiss, cooked, boiled, drained, without salt [175 gram (1.0 cup, chopped)]	150
Nuts, pistachio nuts, raw [123 gram (1.0 cup)]	149
Beans, white, mature seeds, canned [262 gram (1.0 cup)]	134
Nuts, pecans [109 gram (1.0 cup, chopped)]	132
Lima beans, immature seeds, cooked, boiled, drained, without salt [170 gram (1.0 cup)]	126
Beans, black, mature seeds, cooked, boiled, without salt [172 gram (1.0 cup)]	120
Quinoa, cooked [185 gram (1.0 cup)]	118
Milk, dry, whole, without added vitamin D [128 gram (1.0 cup)]	109
Figs, dried, uncooked [149 gram (1.0 cup)]	101
Beans, navy, mature seeds, cooked, boiled, without salt [182 gram (1.0 cup)]	96
Beans, black turtle, mature seeds, cooked, boiled, without salt [185 gram (1.0 cup)]	91
Rice, brown, medium-grain, cooked [195 gram (1.0 cup)]	86
Beans, kidney, California red, mature seeds, cooked, boiled, without salt [177 gram (1.0 cup)]	cup 85
Potatoes, white, flesh, and skin, baked [1.0 potato large (3" to 4-1/4" dia)]	81
Chickpeas (garbanzo beans, Bengal gram), mature seeds, cooked, boiled, without salt [164 gram (1.0 cup)]	79
Millet, cooked [174 gram (1.0 cup)]	77

Kale, scotch, cooked, boiled, drained, without salt [130.gram (1.0 cup, chopped)]	74
Lentils, mature seeds, cooked, boiled, without salt [198 gram (1.0 cup)]	71
Avocados, raw, California [230 gram (1.0 cup, pureed)]	67
Chocolate, dark, 70-85% cacao solids [28.35 gram (1.0 oz)]	65
Sweet potato, cooked, boiled, without skin, with salt [328 gram (1.0 cup, mashed)]	59
Raisins, seedless [165 gram (1.0 cup, packed)]	53
Fish, tuna, light, canned in oil, drained solids [146 gram (1.0 cup, solid or chunks)]	45

Adapted from the United States Department of Agriculture (USDA) National Nutrient Database. Available from https://ods.od.nih.gov/pubs/usdandb/Magnesium-Content.pdf

TABLE 6
Magnesium Requirement According to Institute of Medicine [77]

Age group	Magnesium RDA (mg per a day)
Male	
19 – 30 years	400
31 – 50 years	420

Adapted from National Institutes of Health. Website. Available from https://ods.od.nih.gov/factsheets/Magnesium-healthProfessional/

TABLE 7
Magnesium Requirement According to Indian Council of Medical Research[58]

Age group	Magnesium RDA (mg per a day)
Men (≥ 18 years)	340

Adapted from Indian Council of Medical Research. Website. Available from http://icmr.nic.in/final/RDA-2010.pdf

2.5 The Difference in Body Composition Between Different Ethnicities

BMI is widely used as a surrogate measure to define overweight and obesity and estimate fat mass (FM) because of its simplicity and practicality. A higher BMI associated with a higher likelihood of developing chronic diseases such as type 2 diabetes and cardiovascular diseases. BMI correlates positively with FM. As the FM increases, BMI increases, too because of the increase in the body weight [78]. Based on this assumption, the fat-free mass is being ignored in the BMI calculation. A higher BMI might indicate a higher muscle mass, bone mass or FM mass, not just higher in FM. Interestingly, the body composition varies between ethnicities; therefore, the relationship between the BMI and percentage body fat (%BF) differs. The reasons for the discrepancy in the relationship are the difference in frame size, bone mass, and muscle mass between subjects from different ethnic groups.

In general, Asians have higher %BF compared to their Caucasian counterparts. With a certain %BF, Asians have lower BMI by 3 to 4 points compared to the BMI for his/her Caucasian counterpart[78]. Within Asians, the %BF fat is not similar, too. Asian

Indians have the highest %BF compared to Chinese and Malays[79]. Therefore, many researchers investigated the difference in %BF between Asian Indian men and women compared to other ethnicities. Rush et al. found that Asian Indian men had a higher %BF than Europeans after adjusting for the weight and height (p-value = 0.02). With 29% as %BF of 29, the expected BMI was 30 kg/m^2 in Europeans while the expected BMI in Asian Indians was 25 kg/m^2 [26]. High FM was seen in Asian Indian females compared Europeans [80,81]. The tendency of higher FM in Asian Indians has been detected in the infancy. Asian Indian infants born in the United Kingdom had higher subscapular skinfolds compared to their White counterparts (p-value = 0.02). Stanfield and colleagues speculated that the higher subscapular skinfolds might be an indicator of the tendency to develop central obesity in later life in Asian Indians [82].

On the other hand, fat-free mass was lower in Asian Indian adults[26,80,81] and infants[82] compared to Caucasians. Appendicular Skeletal Muscle Mass (ASMM) is widely used to calculate the skeletal mass from the whole body DXA scan[26,80,81]. Lower muscle mass has been reported in Asian Indian men[26] and women [80,81]. Ultimately, the Asian Indians have higher %BF compared to other ethnicities at any given BMI. World Health Organization (WHO) has endorsed a BMI cut-off for Asian population to accommodate for the variation in %BF and the higher tendency of Asians to develop type 2 diabetes at a normal BMI category **Table 8** [83].

TABLE 8
BMI for Asian Population Compared to International WHO Cut-off [83]

BMI strata	WHO BMI cut-off	Asian BMI cut-off
Normal	18.5 – 24.9	18.5 – 22.9
Overweight	25.0 – 29.9	23.0 – 27.4
Obese class I	30.0 – 34.9	27.5 – 32.4
Obese class II	35.0 – 39.9	32.5 – 37.4
Obese class III	≥ 40.0	≥ 37.5

Adapted from World Health Organization (WHO) Expert Consultation, 2004.

2.6 The Relationship Between Body Composition and Areal Bone Mineral Density

2.6.1 The Relationship Between Lean Mass and Areal Bone Mineral Density

Many studies have been conducted to investigate the association between lean mass (LM) and areal bone mineral density ($_a$BMD) in different ethnic groups and stages of life. Almost all the studies found that LM has a positive association with $_a$BMD **Table 9**. Of these studies, some authors concluded that LM correlated better with $_a$BMD than fat mass and suggested that increasing LM to achieve PBM during young age or maintain $_a$BMD during adulthood.

2.6.1.1 The Mechanism of Interaction Between Lean Mass and Areal Bone Mineral Density

The interaction between LM and bone is complex and involves many levels. Most importantly, the relationship between bone and LM starts with the mechanical load of the body weight. An individual's weight composes of the bone, muscle mass, and fat mass [84]. FM and LM both contribute to the gravitational load on the bone[85]. The kind of load is

called the static load, which is a result of higher body weight in general [86]. Accordingly, the bone will accommodate to this kind of stress in sake for stronger bone that can resist the load exerted on it [87]. LM by itself also responsible for imposing a dynamic/ continuous load on the skeleton during muscle contraction [85,86]. It has been believed that LM has a superior role on bone through the mechanical load since FM contributes by a small percent of body weight compared to LM. It has been estimated the contribution of the fat mass to total body weight in White men is16%[43] - 27%[44].

Secondly, the relationship between LM and bone shares a genetic basis. Osteoblast and myocytes share the same cell precursor. They both differentiate from the mesenchymal stem cells [88]. Also, there is pleiotropy between LM and bone. In other words, a single gene modifies the characteristics of both LM and bone simultaneously. Examples of these genes are GDF-8, MEF-2C, PGC-1alpha[89]. The effect of aging on LM and bone quality is a noticeable characteristic of the genetic interaction between LM and bone. The two systems are affected by aging in the same way. Sarcopenia, which is a decline in muscle mass, and osteoporosis are both results of ageing[88].

Finally, external factors such as sex hormones, estrogens and androgens, exert an impact on bone and muscle mass in the same manner. For example, the decline in serum testosterone concentration, which is an inevitable hallmark of aging, associated with a loss in $_{a}$BMD and bone quality, and muscle mass and strength [89-91]. Amory and colleagues have shown that the administration of testosterone to seniors aged 65 years and older who had low serum testosterone concentrations improved their $_{a}$BMD at the

lumbar spine (L_1–L_4) and total hip compared to placebo group over the study period of three years[92].

TABLE 9
Summary of the Relationship Between Lean Mass and Areal Bone Mineral Density

Authors	Study subjects	Variables	Major finding
Douchi et al. [93]	134 postmenopausal Japanese women (45 exercising women and 89 sedentary women) aged 50 to 60 yrs.	$_a$BMD (DXA) at the LS (L_2 – L_4) LM (kg) (DXA)	LM positively associated with $_a$BMD in the two groups. But the strength of the association was higher in physically active women (r= .415, p-value <0.01) than in sedentary women (r=0.228, p <0.05)
Palmer et al. [85]	72 healthy men aged 20 to 81 yrs.	$_a$BMD (DXA) at LS (L_2 – L_4), left FN, trochanter, and total hip and WB. LM (kg) and left leg LM (kg) (DXA)	LM, and leg LM positively associated with $_a$BMD all measured sites (all p <0.05).
Cheng et al. [94]	1465 and 1534 healthy Chinese men and women, respectively aged 20 to 96 yrs.	$_a$BMD (DXA) at the LS (L_2 – L_4), left FN and WB. LM (Kg) (DXA)	LM positively associated with $_a$BMD at all measured sites in men and women (all p <0.01)
Shin et al. [95]	3945 Korean men aged 20 years and older	$_a$BMD (DXA) at the LS (L_1 – L_4), FN and WB. LM (Kg) (DXA)	LM positively associated with $_a$BMD at all measured sites (all p <0.01)
Bogl et al. [96]	301 Finnish adults (154 men and 147	$_a$BMD (DXA) at the WB.	LM positively associated with $_a$BMD at WB in men

	women) aged 23 to 31 yrs.	LM (kg) (DXA)	and women after adjustment for height (all $p < 0.01$)
Park et al. [97]	1782 Korean adults (762 men and 1020 women) aged 30 yrs. and older	$_a$BMD (DXA) at the WB. LM (kg) (DXA)	LM positively associated with $_a$BMD at WB in men and women after adjustment for height others variables (all $p < 0.05$)
Sotunde et al.[98]	189 Urban black South African women aged 43 yrs. and older	$_a$BMD (DXA) at FN, total hip of non-dominant side and LS ($L_1 – L_4$) LM (kg) (DXA)	LM positively associated with $_a$BMD at the three sites after including FM, age and other variables in the regression model (all $p < 0.01$)
Wang et al. [99]	951 women (317 African American, 154 Asians, 322 Caucasians, 128 Latinas) aged 20 to 25 yrs.	$_a$BMD (DXA) at the WB, LS ($L_2 – L_4$) and left FN LM (kg) (DXA)	LM positively associated with $_a$BMD at WB, LS, and FN after controlling for ethnicity, age, FM and other covariates (all $p < 0.05$)
EL Hage et al. [100]	70 Lebanese men ages 65 to84 yrs.	$_a$BMD (DXA) at the WB, total hip, FN, Ultra-distal radius and 33% radius LM (kg) (DXA)	LM positively associated with $_a$BMD at all measured sites after controlling for age, height and FM (all $p < 0.05$)
EL Hage et al. [101]	110 postmenopausal Lebanese women aged 65 to 84 yrs.	$_a$BMD (DXA) at the WB, LS (L_1–L_4), total hip, FN, Ultra-distal radius and 33% radius LM (kg) (DXA)	LM did not associate with $_a$BMD at WB, total hip and FN after including fat mass and year since menopause in the regression model (all $p > 0.05$)
Kirchengast and Huber [102]	282 healthy older adults (130 men	$_a$BMD (DXA) at the WB and FN	LM positively associated with $_a$BMD at WB and FN

	and 152 women) aged 60 – 92 yrs.	LM (kg) (DXA)	after controlling for age, body weight and FM in men (all $p < 0.01$), but not in women (all p-value > 0.05)
Zhao et al. [103]	1085 Caucasian adults (538 men and 547 women) and 1988 Chinese adults (1110 men and 878 women) aged 19 yrs. and older	$_a$BMD (DXA) at the WB, FN and LS ($L_1 – L_4$) LM (kg) (DXA)	LM positively associated with $_a$BMD at WB, FN and LS after controlling for body weight, age and other variables in Caucasians and Chinese adults (all $p < 0.05$)
Gómez-Cabello et al. [104]	223 seniors (64 men and 159 women) aged 65 to 92 yrs.	$_a$BMD (DXA) at the WB, total hip, FN and LS ($L_1 – L_4$) LM (kg) (DXA)	LM had positive associations with $_a$BMD at all measured sites after controlling for age, FM, height and physical activity in women (all p-value ≤ 0.01). In men, the positive association was only in LS and WB (all $p < 0.05$)
Ho-Pham et al. [105]	210 postmenopausal women aged 50 to 85 yrs.	$_a$BMD (DXA) at the WB, FN and LS ($L_1 – L_4$) LM (kg) (DXA)	LM positively associated with $_a$BMD at all measured sites after controlling for age and FM (all $p < 0.05$).
Choi et al. [106]	461 Korean adults (295 men and 166 women) aged 21 to 83 yrs.	$_a$BMD (DXA) at the total hip, FN and LS ($L_1 – L_4$) LM (kg) (bioelectrical impedance analysis)	LM positively associated with $_a$BMD at all measured sites in men and women after controlling for body weight (all $p < 0.05$)

Taes et al. [107]	677 healthy men aged 25 to 45 yrs.	$_{a}$BMD (DXA) at the WB, total hip and LS ($L_1 – L_4$) LM (kg) (DXA)	LM positively associated with $_{a}$BMD at all measured sites after controlling for either body weight or FM (all $p \leq 0.01$)
Marwaha et al. [108]	1,045 Healthy Asian Indian women	$_{a}$BMD (DXA) at the WB, total hip, FN, LS ($L_1 – L_4$) and 33 % radius LM (kg) (DXA)	LM positively associated with $_{a}$BMD at 33 % radius, WB and total hip after controlling for age, FM, serum 25OHD and PTH (all $p < 0.05$)
Hind et al. [109]	352 (152 men and 190 women)	$_{a}$BMD (DXA) at the left FN, and LS ($L_1 – L_4$) LM (Kg) (DXA)	LM positively associated with LS $_{a}$BMD in men only after controlling for body weight ($p < 0.05$)

Abbreviation: $_{a}$BMD; areal bone mineral density, DXA; dual-energy x-ray absorptiometry, LS; lumbar spine, LM; lean body mass, FN; femoral neck, WB; whole body, FM; fat mass, 25OHD; 25-hydroxyvitamin D, PTH; Parathyroid hormone.

2.6.2 The Relationship Between Fat Mass and Areal Bone Mineral Density

An individual's weight composes of the bone, muscle mass and fat mass [84].Fat mass has positive contributions to the $_{a}$BMD through the mechanical load of body weight on the skeleton (the static load). Also, fat mass is responsible for the productions of certain hormone such as estrogen and leptin that promote bone health. On the other hand, fat mass is associated with an increase in inflammation and inflammatory cytokines that promote bone resorption such as Interleukin (IL-1) and tumor necrosis factor (TNF)[86]. Because of the complex relationship, the associations between fat mass and the $_{a}$BMD

been examined in previous research studies were different **Table 10**. Some researchers found the association between fat mass and $_a$BMD is a sex-dependent and age-dependent. In other words, a positive association was seen in postmenopausal women and older men, while the negative association observed in younger populations.

TABLE 10
Summary of the Relationship Between Fat Mass and Areal Bone Mineral Density

Authors	Study subjects	Variables	Major finding
Douchi et al. [93]	134 postmenopausal Japanese women aged 50 to 60 yrs. (45 exercising women and 89 sedentary women)	$_a$BMD (DXA) at the LS ($L_2 – L_4$) FM (kg) (DXA)	FM positively associated with $_a$BMD only in sedentary women ($r=.251$, $p <0.05$)
Cheng et al. [94]	1465 and 1534 healthy Chinese men and women, respectively aged 20 to 96 yrs.	$_a$BMD (DXA) at the LS ($L_2 – L_4$), left FN and WB. FM (kg) and %BF (%) (DXA)	FM negatively associated with $_a$BMD at the LS in young men and positively with $_a$BMD all measured sites in older men (all $p <0.01$) FM positively associated with $_a$BMD at most of the measured sites in women (all $p <0.01$) %BF negatively associated with $_a$BMD at most of the measured sites in men (all $p <0.01$)

			%BF negatively associated with $_{a}$BMD in young women and positively in postmenopausal women (all $p < 0.05$).
Shin et al. [95]	3945 Korean men aged 20 yrs. and older	$_{a}$BMD (DXA) at the LS ($L_1 – L_4$), FN and WB. FM (kg), %BF (%) (DXA)	FM and %BF negatively associated with $_{a}$BMD at all measured sites (all $p < 0.01$) after adjustment for body weight.
Bogl et al. [96]	301 Finnish adults (154 men and 147 women) aged 23 to 31 yrs.	$_{a}$BMD (DXA) at the WB. FM (kg) (DXA)	FM positively associated with $_{a}$BMD at WB in both men and women after adjustment for height (all $p < 0.05$)
Park et al. [97]	1782 Korean adults (762 men and 1020 women) aged 30 yrs. and older	$_{a}$BMD (DXA) at the WB. FM (kg) (DXA)	FM positively associated with $_{a}$BMD at WB in both men and women after adjustment for height others variables (all $p < 0.05$)
Sotunde et al.[98]	189 Urban black South African women aged 43 yrs. and older	$_{a}$BMD (DXA) at FN, total hip of non-dominant side and LS ($L_1 – L_4$) FM (Kg) (DXA)	FM did not associate with $_{a}$BMD at three sites after including LM, age and other variables in the regression model (all $p > 0.05$)
Wang et al. [99]	951 women (317 African American, 154 Asians, 322 Caucasians, 128 Latinas) aged 20 to 25 yrs.	$_{a}$BMD (DXA) at the WB, LS ($L_2 – L_4$) and left FN FM (Kg) (DXA)	FM positively associated with $_{a}$BMD at WB, LS, and FN after controlling for ethnicity, age, LM and other variables (all $p < 0.05$)

EL Hage et al. [100]	70 Lebanese men ages 65 to 84 yrs.	$_a$BMD (DXA) at the WB, LS ($L_2 – L_4$), total hip, FN, Ultra-distal radius and 33% radius FM (Kg) and %BF (%) (DXA)	FM did not associate with $_a$BMD at all measured sites after controlling for age, height and LM (all $p > 0.05$)
EL Hage et al. [101]	110 postmenopausal Lebanese women aged 65 to 84 yrs.	$_a$BMD (DXA) at the WB, LS (L_1–L_4), total hip, FN, Ultra-distal radius and 33% radius FM (Kg) (DXA)	FM positively associated with $_a$BMD at WB, total hip and FN after including LM and year since menopause in the regression model (all $p \leq 0.01$)
Kirchengast and Huber [102]	282 healthy older adults (130 men and 152 women) aged 60 to 92 yrs.	$_a$BMD (DXA) at the WB and FN FM (Kg) (DXA)	FM positively associated with $_a$BMD at WB and FN after controlling for age, body weight and LM in women (all $p < 0.01$), but not in men (all $p > 0.05$)
Zhao et al. [103]	1085 Caucasian adults (538 men and 547 women) and 1988 Chinese adults (1110 men and 878 women) aged 19 years and older	$_a$BMD (DXA) at the WB, FN and LS ($L_1 – L_4$) FM (Kg) and %BF (%) (DXA)	FM and %BF negatively associated with $_a$BMD at WB, FN and LS after controlling for body weight, age and other variables in Caucasians and Chinese adults (all $p < 0.01$)
Gómez-Cabello et al. [104]	223 seniors (64 men and 159 women) aged 65 to 92 yrs.	$_a$BMD (DXA) at the WB, total hip, FN and LS ($L_1 – L_4$) FM (Kg) (DXA)	FM negatively associated with $_a$BMD at the WB after controlling for age, LM,

			height and physical activity in men ($p < 0.01$)
Ho-Pham et al. [105]	210 postmenopausal women aged 50 to 85 yrs.	$_a$BMD (DXA) at the WB, FN and LS ($L_1 – L_4$) FM (Kg) (DXA)	FM positively associated with $_a$BMD at FN and LS after controlling for age and LM (all $p < 0.05$).
Choi et al. [106]	461 Korean adults (295 men and 166 women) aged 21 to 83 yrs.	$_a$BMD (DXA) at the total hip, FN and LS ($L_1 – L_4$) FM (kg) (bioelectrical impedance analysis)	FM negatively associated with $_a$BMD at all measured sites in men, except for total hip in men and women after controlling for body weight (all $p < 0.05$)
Taes et al. [107]	677 healthy men aged 25 to 45 yrs.	$_a$BMD (DXA) at the WB, total hip and LS ($L_1 – L_4$) FM (kg) (DXA)	FM negatively associated with $_a$BMD at all measured sites after controlling for either body weight or LM (all $p \leq 0.01$)
Hind et al. [109]	352 (152 men and 190 women)	$_a$BMD (DXA) at the left FN and LS ($L_1 – L_4$) FM (kg) (DXA)	FM did not associate with $_a$BMD either in men or women after controlling for body weight (all $p > 0.05$)

Abbreviation: $_a$BMD; areal bone mineral density, DXA; dual-energy x-ray absorptiometry, LS; lumbar spine, FM; fat mass, FN; femoral neck, WB; whole body, LM; lean body mass, % BF; percent body fat.

2.6.3 The Relationship Between Visceral Adipose Tissue and Subcutaneous Adipose with Areal Bone Mineral Density

In recent years, the attention has shifted from total body fat toward fat distribution in the body because abdominal/central adiposity had been associated with insulin resistance

and increase the risk of cardiovascular disease[110] rather than the total body fat. Abdominal fat contains visceral fat, or intra-abdominal fat, and subcutaneous fat. The visceral Adipose tissue is in the mesentery and omentum regions. This kind of fat is completely different from subcutaneous adipose tissue regarding secretion of hormones and peptides, function, adipocytes size, and blood vascularity and innervations. Due to these differences, visceral adipose tissue is a risk factor for insulin resistance, type 2 diabetes and cardiovascular diseases[110]. The relationship between bone density and fat had been dedicated to investigating the difference between the visceral adipose tissue and subcutaneous adipose tissue with bone density.

The association between visceral adipose tissue and subcutaneous adipose tissue with $_{a}$BMD has been investigated in adults, adolescents, diabetic subjects and in different ethnic groups. The findings, however, are inconsistent. The findings can be categorized into negative association[106,111-114] and lack of association[109,115] between visceral adipose tissue and bone density **Table 11**, while the range of finding is higher in the association between subcutaneous adipose tissue and $_{a}$BMD **Table 12**. The positive association seen in many studies[106,114] between visceral adipose tissue and $_{a}$BMD was eliminated or became negative after controlling for some confounders such as body weight and age. This shows the statistical handling of the data could be a source of the variation in findings. The method as well selected to calculated visceral adipose tissue could play a significant role in the inconsistency. Salimzadeh and colleague [115] used the bioelectrical impedance analysis (BIA) to estimate the visceral adipose tissue. In fact, the model of BIA used in that study only estimates the trunk fat percentage. Abdominal fat contains

both visceral adipose tissue and subcutaneous adipose tissue [116]. As a result, the results of the study did not investigate the genuine relationship between visceral adipose tissue and $_{a}$BMD accurately.

TABLE 11
Summary of The Relationship Between Visceral Adipose Tissue and Areal Bone Mineral Density

Authors	Study subjects	Variables	Major finding
Salimzadeh et al. [115]	95 overweight and obese women aged 30 to 50 yrs.	$_{a}$BMD (DXA) at the LS ($L_2 – L_4$) and FN VAT (%) (bioelectrical impedance analysis)	VAT did not associate with $_{a}$BMD either at LS or FN after controlling for age, marital status, number of children and occupation (all $p > 0.05$)
Campos et al. [111]	125 obese adolescents (45 boys and 80 girls) aged 14 to 18 yrs.	$_{a}$BMD (DXA) at the WB VAT (cm) (Abdominal ultrasound)	VAT negatively associated with $_{a}$BMD at spine only in boys ($p = 0.03$)
Choi et al. [106]	461 Korean adults (295 men and 166 women) aged 21 to 83 yrs.	$_{a}$BMD (DXA) at the total hip, FN and LS ($L_1 – L_4$) VAT (cm^2) (Computed tomography)	VAT negatively associated with $_{a}$BMD at all measured sites in men and women after controlling for body weight, age and other variables (all $p < 0.05$)
Hind et al. [109]	352 (152 men and 190 women)	$_{a}$BMD (DXA) at the left FN and LS ($L_1 – L_4$) VAT (gm) (DXA)	VAT did not associate with $_{a}$BMD in men or women after controlling for body weight ($p > 0.05$)

Russell et al. [112]	30 girls (15 obese and 15 normal weight) aged 12 to 18 yrs.	$_{a}$BMD (DXA) at the WB, total hip and LS VAT (cm^2) (magnetic resonance imaging)	VAT negatively associated with $_{a}$BMD at the WB in the whole sample and with $_{a}$BMD at the LS only in obese girls (all p =0.04)
Katzmarzyk et al. [113]	1081 adults (444 African American men, and women and 637 White men and women) aged 18 to 74 yrs.	$_{a}$BMD (DXA) at the WB VAT (cm^2) (Computed tomography)	VAT negatively associated with $_{a}$BMD at the WB after controlling for age, LM and SAT in all groups except for African American women and White men (all $p < 0.05$)
Yamaguchi et al. [114]	312 diabetic Japanese adults (187 men [aged 28 to 83 yrs.] and 125 postmenopausal women [aged 46 to 82 yrs.])	$_{a}$BMD (DXA) at the WB, LS, FN and 33% radius VAT (cm^2) (Computed tomography)	VAT negatively associated with $_{a}$BMD at WB and 33% radius in women only after controlling for body weight (all $p < 0.05$)

Abbreviation: $_{a}$BMD; areal bone mineral density, DXA; dual-energy x-ray absorptiometry, LS; lumbar spine, FN; femoral neck, VAT; visceral adipose tissue, WB; whole body, SAT; subcutaneous adipose tissue

TABLE 12
Summary of the Relationship Between Subcutaneous Adipose Tissue and Areal Bone Mineral Density

Authors	Study subjects	Variables	Major finding
Campos et al. [111]	125 obese adolescents (45	$_{a}$BMD (DXA) at the WB	SAT positively associated with $_{a}$BMD at lower limb in boys ($p < 0.01$)

	boys and 80 girls) aged 14 to 18 yrs.	SAT (cm) (Abdominal ultrasound)	
Choi et al. [106]	461 Korean adults (295 men and 166 women) aged 21 to 83 yrs.	$_{a}$BMD (DXA) at the total hip, FN and LS (L_1 – L_4) SAT (cm^2) (Computed tomography)	SAT negatively associated with $_{a}$BMD at LS only in men after controlling for body weight, age and other variables (p-value =0.035)
Russell et al. [112]	30 girls (15 obese and 15 normal weight) aged 12 to18 yrs.	$_{a}$BMD (DXA) at the WB, total hip and LS SAT (cm^2) (magnetic resonance imaging)	SAT positively associated with $_{a}$BMD at the WB and LS in the whole sample (all $p < 0.05$)
Katzmarzyk et al. [113]	1081 adults (444 African American men, and women and 637 White men and women)	$_{a}$BMD (DXA) at the WB SAT (cm^2) (Computed tomography)	SAT did not associate with WB $_{a}$BMD after controlling for age, LM and VAT in any of the four groups (all $p > 0.05$)
Yamaguchi et al. [114]	312 diabetic adults (187 men [aged 28 to 83 yrs.] and 125 postmenopausal women [aged 46 to 82 yrs.])	$_{a}$BMD (DXA) at the WB, LS, FN, 33% radius SAT (cm^2) (Computed tomography)	SAT negatively associated with $_{a}$BMD at WB and FN in men and 33% radius in women after controlling for body weight (all $p < 0.05$)

Abbreviation: $_{a}$BMD; areal bone mineral density, DXA; dual-energy x-ray absorptiometry, SAT; subcutaneous adipose tissue, FN; femoral neck, LS; lumbar spine, WB; whole body, VAT; visceral adipose tissue.

CHAPTER 3: PROTOCOL AND METHODS

3.1 Protocol

3.1.1 Participants

3.1.1.1 Inclusion criteria

A participant who fulfilled all the following requirements was eligible to take part in the study:

1. Male who was either Asian Indian or Caucasian
    a. Asian Indian participants were defined themselves as natives of first or subsequent generation immigrants who can trace their ancestry to any country of the Indian subcontinent. These countries are India, Bangladesh, Nepal, Pakistan and Sri Lanka.
    b. Caucasian participants were defined themselves as non-Hispanic who did not belong to any other ethnic group
2. Age between 20 to 50 years
3. Free of the chronic diseases or medications that might affect bone metabolism
4. Signed informed consent

3.1.1.2 Exclusion criteria:

A participant who had health conditions, or on medications that might impact his body composition or bone outcomes was not eligible for the study. The exclusion criteria were:

1. Participants with cancer or cancer therapy in the past six months
2. Participants with kidney disease or kidney stones in the past year

3. Participants with immune disease or on steroid drugs to suppress immunity
4. Participants with unmanaged thyroid disease
5. Participants with a heart attack or stroke within the past year
6. Participants with arthritis taking prednisone
7. Participants with liver disease
8. Participants on osteoporosis medication

3.1.2 Study Design

This was a cross-sectional study with a single visit. The entire visit did not take more than 2 hours. The timeline of the study is illustrated below **Figure 3**. The data that was collected during the visit will be explained in a data collection section.

Figure 3: Study Timeline

3.1.3 Study Groups

The study had two groups **Figure 4**. The first group was composed of Asian Indian men. The second group was composed of Caucasian participants

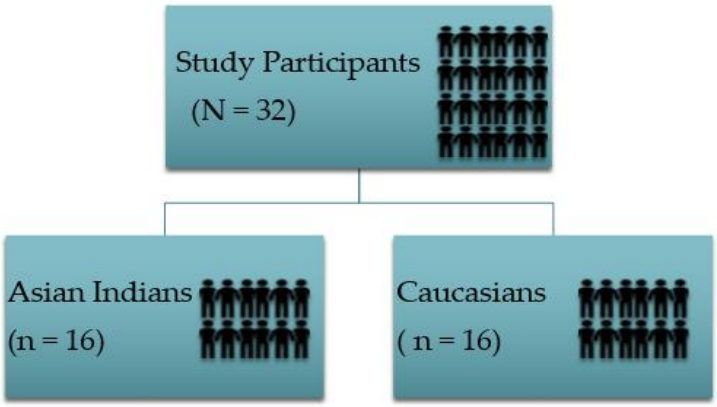

Figure 4: Study Groups

3.1.4 Sampling Techniques

Convenience sampling was the type of sampling method for the study. The sample of the study was composed of individuals who were interested in and eligible for participating in the study.

3.1.5 Research Location and Space

The bone lab on the third floor of 3 Parkway Building (1601 Cherry St. Philadelphia, PA 19102) was the location, to which the participants came. At this place, informed consent was signed by the participant, and research assistants collected information about anthropometrics measurements and dietary intakes using food frequency questionnaires and 24-hour recall. The second location was a room (203) on the second floor of 3 Parkway Building (1601 Cherry St. Philadelphia, PA 19102) for doing the DXA scans. A research assistant was responsible for escorting the subject to move from the third to second floor.

3.1.6 Recruitment

The recruitments of participants for the study was through research flyers that were disseminated to Drexel University's University City and Center City Campus **Appendix 1** and via word-of-mouth. The flyers were proved by the Institutional Review Board (IRB). The flyer contained information about the study written in simple language. The research objective, eligibility criteria, compensation fee and the email address of the project and phone number and location of the lab were the information in the flyer. Interested participants called the lab phone number or sent an email to the email project, which were in the flyers.

3.1.7 Screening

A research assistant conducted a telephone eligibility questionnaire **Appendix 2** for participants who were interested in the study. During the phone call, details explaining the study's objective, procedures and the duration of the visit were discussed with the participant. The research assistant confirmed the participant's eligibility by asking for a brief medical history form, medications and treatments currently being used, and no exclusion criteria present. Once the participant agreed to participate in the study, an in-person appointment was scheduled at the Bone Lab (1601 Cherry Street Room 317, Philadelphia, PA 19102). Each participant received $30 monetary compensation at the end of his visit to incentivize people to participate in the study.

3.2 Data Collection

3.2.1 Informed Consent Form

The study participant arrived at the laboratory, and a research assistant began with the informed consent process **Appendix 3**. The research assistant gave time for the participant to read the informed consent at his own pace. After that, the research assistant verbally explanation major points in the informed consent including the principal investigator and institutional review board contact information and the objectives of conducting the study. Two identical copies of the informed consent were signed and dated by the research assistant and the participant. One copy of the signed informed consent was given to the participant, while the other kept in the participant's file.

3.2.2 Demographics

Participant's demographic information including information about age, ethnicity, contact information and tobacco and alcohol consumptions and others was collected during the visit. The information was self-reported by the participants **Appendix 4**.

3.2.3 Medical History

The participant was asked to complete a medical history form **Appendix 4**. The form had questions about disease history, tobacco, and alcohol use, eating disorder diagnosis, recent and current medications and supplements intake, any medical comments, and any recent weight loss/gain greater than 10 lbs.

3.2.4 Anthropometrics Measurements

3.2.4.1 Weight and Height

A stadiometer with a balance beam scale (Seca 700 Physician's Balance Beam Scale, Chino, CA, USA) was used to measure the height (Inches) and weight (Pounds [lbs.]) while participants were with minimal and light clothes, without shoes, and without anything heavy in his pocket. One of the research assistants administered the measurements procedures, while the other research assistant recorded the data **Appendix 5**. The weight and height were reported to the nearest 0.25 lb and 0.25 inches, respectively.

3.2.4.2 Waist Circumference

Using a non-stretchable measuring tape (Health Mobius® Circumference (Girth) measuring Tape-Body Tape Measure), a research assistant took three measurements of participant's waist circumference in inches. One of the research assistants administered the measurements procedures, while the other research assistant recorded the data **Appendix 5**. The measurements were taken around trunk one inch above the umbilicus. The average of the three measurements took and reported to the nearest 0.25 inches.

3.2.5 Dietary Information and Physical Activity

3.2.5.1 Magnesium Food Frequency Questionnaire (Mg-FFQ)

A magnesium food frequency questionnaire (Mg-FFQ) **Appendix 6** was developed by the principal investigator of this study. The principal investigator and her undergraduate seniors in Nutrition Department at Drexel University are conducting research to validate the MgFFQ against fourteen days food diary record[117]. The Mg-FFQ

contained a list of 33 food items that have magnesium content ranged from 6 mg to 178 mg per serving. Participants were asked to approximately quantify the number of servings and frequency of consumption per day, week, or month. Visual demonstrations of the measuring cups were provided to guide the participant in the process of specifying the amount.

FFQs are widely used in the research to assess the intake of specific food groups or nutrients. A review by Cade et al. demonstrated that 166 out of 223 studies included in the review, about 74% of the studies, used the FFQ to assess the consumption of nutrients [118]. It has been found that FFQs are considered a practical tool to evaluate the dietary intake [119].

3.2.5.2 Calcium Food Frequency Questionnaire (Ca-FFQ)

A calcium food frequency questionnaire (Ca-FFQ) **Appendix 7** was taken from "calcium calculator" by International Osteoporosis Foundation website [120], which was developed by a pharmaceutical company, called Takeda. The Ca-FFQ contained 80 food items, and the content of calcium ranged from 7 mg to 445 mg per serving. Participants were asked to approximately quantify the number of servings and frequency of consumption per day, week, or month. Visual demonstrations of the measuring cups were provided to guide the participant in the process of specifying the amount.

3.2.5.3 Twenty-Four Hour Dietary Recall

A research assistant administered twenty-four-hour diet recall **Appendix 8** to calculate energy and macronutrients. The participants were asked to remember and report his food intake for the day preceding the day of the visit to the best of his abilities. The

research assistant followed the 5-Step Multiple-Pass Approach. The analysis of the twenty-four-hour diet recall was completed using the foodWorks software (FoodWorks ® version 17 Copyright © 2015) A self-estimated approach of physical activity level was used to quantify the level of physical activity.

3.2.6 Dual Energy X-ray Absorptiometry

In this study, the Dual-energy x-ray absorptiometry (DXA) scan was used to measure areal bone mineral density ($_a$BMD) and body composition of the participants. The use of DXA scan has many advantages encouraging researchers and clinicians to use. The advantages of DXA are 1) low level of radiations a subject gets during the scan compared to Computer Tomography (CT), 2) the easiness of setting up of the subject for the scan, 3) the shortness of the scan [121] and 4) the images have high resolution[122]. There are two beams of X-ray each one had a different energy to give an estimated measure of the bone and soft tissue. The underline principle of DXA is that the absorption of the ionized radiation DXA emits by bone is proportional to the amount of bone[29]. As a result, the dense bone will absorb more radiation than small bone. DXA (Lunar iDXA, enCORE Software Version 17, GE Healthcare, United Kingdom) was used to measure the $_a$BMD. To check the validity and reliability of the DXA, a research assistant calibrated the DXA on the same day and before scanning each participant. A quality control phantom box was used during the calibration process. The level of radiation a participant was exposed to during the scan is minimal, and it had been estimated to be less than the radiation a person will be exposed to during a cross-country flight.

Before doing the scan, the participant was asked to remove all jewelry, metals in his clothes such as a zipper, and remove his shoes. A patient gown with back ties was provided to a participant who had metal on his clothes. The participant was instructed to lie on the DXA bed inside the box printed on the DXA table. The measurements of $_{a}$BMD were taken at the lumbar spine (L_2 - L_4), dual femoral neck, 33% radius (a proximal third of the radius) of the nondominant hand and whole body.

DXA divides the whole-body scan intro three distinct parts, which are bone mineral content, fat mass, and bone-free mass. Also, enCORE Software Version 17 is capable of measuring regional fat distribution in the android region and gynoid region. DXA defines the region of interest (ROI) for android fat as "lower boundary at pelvis cut, upper boundary above pelvis cut by 20% of the distance between pelvis and neck cuts and lateral boundaries are the arm cuts" and for gynoid fat as "upper boundary below the pelvis cut line by 1.5 times the height of the android ROI and lateral boundaries are the outer leg cuts[123]."**Figure 5**

Moreover, enCORE Software Version 17 has been supplemented with software to measure the visceral adipose tissue in the abdominal region or android region. The ability of DXA's enCORE Software to measure visceral adipose tissue had been validated with the measurements of visceral adipose tissue taken by computed tomography in subjects age between 18 to 90 years with BMI between 18.5 to 40 kg/m^2. Person correlations of visceral adipose tissue by DXA and computed tomography were 0.973, 0.979, and 0.978 for men, women, and total sample, respectively [124].

For body composition analysis, the following variables were derived from whole body scan 1) total lean mass (lb), 2) total body fat mass (lb), 3) android fat mass (lb), 4) gynoid fat mass (lb), 5) visceral adipose tissue (lb) and 6) subcutaneous adipose tissue (lb).

DXA calculates the $_a$BMD at any site as $\frac{Bone\ Mineral\ Content\ (gm)}{Projected\ Area\ (cm^2)}$ and $_a$BMD is expresed as (gm/cm^2) [31]. Carter et al. stated that taller subjects will have higher $_a$BMD compared to shorter subjects eventhough they have simlar volumetric bone denisty[29]. Given the fact that Caucasians are taller and hevier than Asias Indain subjects[23,25,27,80,125], $_a$BMD will be lower in Asian Indians becsue of their short stataure. As a way to eliminate that artifact, Carter and collueges have suggested to use Bone Mineral Apparent Density (BMAD) in gm/ cm^3 . BMAD was caluacted as $\frac{aBMD\ (\frac{gm}{cm^2})}{Height\ (m)}$. BMAD is an estimate of the volumetic bone mineral desnity based on $_a$BMD[29].

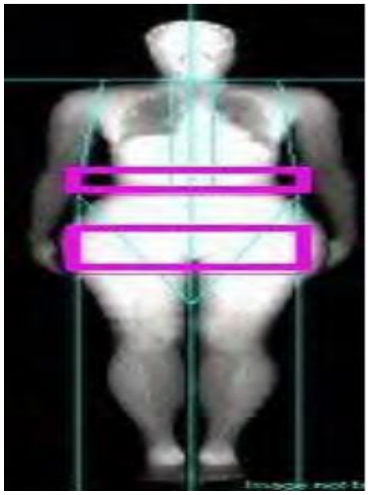

Figure 5: Region of Interest for Android and Gynoid Fat. Adapted from GE Healthcare Lunar. 2016 [123]

3.2.7 Data Management

The documents that were used for collecting the participants' information were stored in a locked drawer for the PI in the secured room which had a security code only known by the research team. The room was on the third floor of 3 Parkway Building (1601 Cherry St. Philadelphia, PA 19102). On a computer that linked to the encrypted server, a research assistant entered the participants' information into a Microsoft® Excel data sheet. Then, Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corp., Armonk, NY, 2016) was used to run the statistical tests.

3.3 Statistical Analysis

Descriptive statistics, including means, standard deviations were calculated for the total sample and Asian Indian and Caucasian men for variables that were normality distributed. For non-normally distributed variables, median and interquartile range were calculated. Because age and BMI have an impact on $_{a}$BMD, the Asian Indians and Caucasians were matched for age and BMI. Since the age and BMI were not normality distributed within ethnic groups, Mann Whitney U test was used to compare our age and BMI between the two groups Non-significant differences between Asian Indians and Caucasians for age and BMI were found, which confirmed that two groups were adequately matched. Data was analyzed using IBM SPSS Statistics software (version 24, IBM Corporation, Armonk, NY).

3.3 Analyses for Specific Aims

3.3.1 Statistical Analyses for Specific Aim 1

The $_{a}$BMD at all skeletal sites were normally distributed in Asian Indians and Caucasians according to Shapiro-Wilk test. Therefore, independent sample t-test was used to determine whether a difference exists in $_{a}$BMD for the whole body, and at the lumbar spine (L_2 -L_4), femoral neck or 33% radius of the non-dominant hand between Asian Indian and Caucasian men matched for age and BMI. The independent variable was ethnicity, which was treated as a dichotomous variable.

3.3.2 Statistical Analyses for Specific Aim 2

Lean mass was normally distributed in Asian Indians and Caucasians according to Shapiro-Wilk test. Therefore, independent sample t-test was used to determine whether a difference exists in total lean mass between Asian Indian and Caucasian men matched for age and BMI. The independent variable was ethnicity, which was treated as a dichotomous variable. However, the gynoid fat mass and android fat mass were not normally distributed in Asians Indians and Caucasians. As a result, Mann Whitney U test was used to determine whether a difference exists in android fat mass or gynoid fat mass between Asian Indian and Caucasian men matched for age and BMI.

3.3.3 Statistical Analyses for Specific Aim 3

Daily calcium and magnesium intakes were not normally distributed in Asians Indians and Caucasians according to Shapiro-Wilk test. As a result, Mann Whitney U test was used to determine whether a difference exists in calcium or magnesium intake between Asian Indian and Caucasian men matched for age and BMI.

3.3.4 Statistical Analyses for Specific Aim 4

In the overall sample, lean mass, $_{a}$BMD at the lumbar spine (L_2- L_4) and $_{a}$BMD at the femoral neck were normally distributed according to Shapiro-Wilk test. The associations between 1) lean mass and $_{a}$BMD at the lumbar spine (L_2- L_4) and 2) lean mass and $_{a}$BMD at the femoral neck were performed using Pearson product-moment correlation coefficients. Android fat mass and gynoid fat mass were not normally distributed in the overall sample; therefore, the associations between 1) android fat mass and $_{a}$BMD at the lumbar spine (L_2- L_4); 2) android fat and $_{a}$BMD at the femoral neck; 3) gynoid fat mass and $_{a}$BMD at lumbar spine (L_2- L_4); and 4) gynoid fat mass and $_{a}$BMD at the femoral neck were performed using Spearman rank order correlation coefficient.

Lean mass, android fat mass and gynoid fat mass were not normally distributed in Asian Indians and Caucasians according to Shapiro-Wilk test; therfore, the assoications between 1) lean mass and $_{a}$BMD at the lumbar spine (L_2- L_4) in Asian indians and Caucasians ; 2) lean mass and $_{a}$BMD at the femoral neck in Asian indians and Caucasians ; 3) android fat mass and $_{a}$BMD at the lumbar spine (L_2- L_4) in Asian indians and Caucasians; 4) android fat and $_{a}$BMD at the femoral neck in Asian indians and Caucasians; 5) gynoid fat mass and $_{a}$BMD at lumbar spine (L_2- L_4) in Asian indians and Caucasians ; and 6) gynoid fat mass and $_{a}$BMD at the femoral neck in Asian indians and Caucasians were perfomed using Spearman rank order correlation coefficient.

For comparing the relationships between body composition variables and $_{a}$BMD in Asian Indian and Caucasian men matched for age and BMI, the

Fisher's *r* to *z* transformation was used. If the Z score ≤ -1.96 or ≥ 1.96, that indicates the two correlations were significantly different at the $p < 0.05$ level.

3.3.5 Statistical Analyses for Exploratory Aim 1

Chi-square contingency table test was used to determine whether a difference exists in the percentage of Asian Indian men who met the daily RDA for calcium established for Asian Indian men by Indian Council of Medical Research and the percentage of Caucasian men who met the daily RDA for calcium established by the Institute of Medicine. The two variables were the ethnicity, either Asian Indian male or Caucasian male, and meeting the criterion of daily calcium RDA, either meeting the RDA or not meeting the RDA.

3.3.6 Statistical Analyses for Exploratory Aim 2

The visceral adipose tissue was normally distributed in Asian Indians and Caucasians according to Shapiro-Wilk test. Therefore, independent sample t-test was used to determine whether a difference exists in visceral adipose tissue between Asian Indian and Caucasian men matched for age and BMI. The independent variable was ethnicity, which was treated as a dichotomous variable. However, subcutaneous adipose tissue was not normally distributed in Asians Indians and Caucasians. Mann Whitney U test was used to determine whether a difference exists in subcutaneous adipose tissue between Asian Indian and Caucasian men matched for age and BMI.

3.3.7 Statistical Analyses for exploratory Aim 3

The BMAD at all skeletal sites were normally distributed in Asian Indians and Caucasians according to Shapiro-Wilk test. Therefore, independent sample t-test was

used to determine whether a difference exists in BMAD for the whole body, at the lumbar spine (L_2 -L_4), femoral neck or 33% radius of the non-dominant hand between Asian Indian and Caucasian men matched for age and BMI. The independent variable was ethnicity, which was treated as a dichotomous variable.

CHAPTER 4: JOURNAL MANUSCRIPT

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INTRODUCTION

Osteoporosis is a public health concern in the United States and around the globe. The burden of osteoporotic fracture entails financial, physical and psychosocial aspects. Annually, approximately $10 to $15 billion is the financial cost that is directed to hospitals' care for subjects with osteoporotic fractures in the United States[1]. With the increase in life expectancy, the proportion of the elderly population is expected to increase. Aging is a risk factor for osteoporosis and hence osteoporotic fractures. During the last three decades, there has been an increase in hip fractures by 2 to 3 times in Asian countries. It has been estimated that more than half of the osteoporotic fractures will occur in Asian countries by 2050 [2]. Even though the prevalence of osteoporosis in men is lower than in women, the expected increase in the percentage of numbers of cases with hip fracture in men is 89% compared to 69% in women during the period between 2000 to 2025 [3]. The mortality rate in men is higher due to osteoporosis and osteoporotic fractures compared to women in developed and developing countries such as India[4,5].

Low areal bone mineral density ($_a$BMD) is considered a strong risk factors for future fracture risk. The $_a$BMD is different between people from different ethnic groups. Asian Indians have lower $_a$BMD compared to Caucasians[6-8]. In fact, body composition and the intake of specific nutrients contribute to the bone mass. For example, lean mass correlated positively with $_a$BMD[9-12], but findings were inconsistent regarding the association between fat mass and $_a$BMD[10-13]. Previous studies have been shown that body composition differs between subjects from different ethnic groups. Asian Indians have higher body fat, and visceral adipose tissue and subcutaneous adipose tissue, but lower

muscle mass compared to Caucasians[14-18]. Regarding the role of diet in bone mass, dietary intake plays a crucial role in optimizing peak bone mass, maintaining of bone mass and protecting against the regular load imposed on bone [19]. One of the well-known nutrients for acquiring and maintaining bone mass is calcium. Even though Asian Indians have lower $_{a}$BMD, their calcium RDA is lower than the RDA established for their Caucasian counterparts[20,21].

The primary aim of the current study was to examine the difference in $_{a}$BMD between healthy Asian Indian immigrants and Caucasians matched for age and body mass index (BMI) using cross-sectional study design. Also, the differences in body composition and dietary intake between the two groups were appraised in the study. In addition, the correlation between lean amass and regional fat distributions with $_{a}$BMD at the lumbar spine and femoral neck were evaluated in the overall sample and within each ethnic group.

SUBJECTS AND METHODS

Subjects

Healthy males who self-identified themselves as either Asian Indians or Caucasians from Philadelphia community were recruited for this cross-sectional study. Participants were healthy and did not have any medical conditions that might interfere with bone or body composition outcomes. Subjects diagnosed with diseases known to interfere with bone metabolism or body composition were excluded. The study was approved by the Institutional Review Board of Drexel University and informed consent was obtained from each participant.

Bone Mass and Density Measurements

For each participant, there were four values of $_{a}$BMD (gm/cm^2) at whole body, lumbar spine (L_2-L_4), dual femoral neck and 33% radius of the non-dominant hand measured by dual-energy X-ray absorptiometry (DXA) (Lunar iDXA, United Kingdom). From the whole-body scan, total lean mass (lb), android fat mass (lb) and gyroid fat mass (lb). Measurements of visceral adipose tissue (lb) and subcutaneous adipose tissue (lb) were recorded for only 12 participants out of 32 because the software used to measure these fat compartments had not installed at the beginning of the study. Participants completed medical history form which induced information about age, medical conditions, medications and supplements taking recently. All scans taken were in accordance with the standard protocol for positioning the participants. Also, quality control procedure was performed according to the guidelines set by DXA manufacturer. The coefficient of variation of repeated measures of the $_{a}$BMD at the lumbar spine, femoral neck and total body is 1.0%[22], which indicates high precision level. The difference in skeletal size between Asian Indians and Caucasians has been considered as one of the possible causes for lower $_{a}$BMD in Asian Indians compared to Caucasians[23-25]. To eliminate that artifact, Carter and colleagues have suggested using Bone Mineral Apparent Density (BMAD) in gm/ cm^3. BMAD was calculated as $_{a}BMD$ (gm/cm^2)/ ($Height$ (m)). BMAD is an estimate of the volumetric bone mineral density based on $_{a}$BMD[26].

Anthropometrics Measurements

Anthropometrics measurements of weight and height were measured while participants were with minimal and light clothes and without shoes to the nearest 0.25 lb and 0.25 inches, respectively using a stadiometer with a balance beam scale (Seca 700 Physician's Balance Beam Scale, USA). Average of three waist circumference measurements around the torso one inch above the umbilicus the nearest 0.25 inch was reported. Body mass index (BMI) was calculated by dividing weight over height and expressed as kg/m^2.

Diet information

Diet history was collected using two food frequency questionnaires (FFQ), one for calcium by International Osteoporosis Foundation[27], while the other for magnesium[28]. The FFQ was used to give an estimate of the consumption of food that is rich in either calcium or magnesium over the past six months. Participants were asked to approximately quantify the number of servings and frequency of consumption per day, week or month. Visual demonstrations of the measuring cups were provided to guide the participant in the process of specifying the amount was consumed. The daily intake of calcium and magnesium were calculated from FFQs. Additionally, 24-hour food recall was administered to calculate energy and macronutrients. The food recall was analyzed using FoodWork software (FoodWorks ® version 17 Copyright © 2015)

Statistical analysis

Data was analyzed using SPSS 24 (IBM Corp., Armonk, NY, 2016). Means and SDs were calculated for all continuous variables of anthropometric measurements, bone density, body composition and nutrient intake that were normally distributed and median and interquartile range (IQR) for non-normally distributed variables. The level of significance was set at P 0.05 in 2-tailed testing, which is a commonly used value in previous similar research. The differences between Asian Indians and Caucasians in all continuous variables were tested by performing Independent sample t-test for normally distributed variables and Mann-White U for non-normally distributed outcomes. For comparing the difference in the percentages of categorical variables between Asian Indians and Caucasians, Chi-square test contingency table was performed. To test the association between the body composition variables and bone density at the lumbar spine and femoral neck, Pearson product-moment correlation coefficient was used for normally distributed variables and Spearman's rank correlation coefficient when Pearson product-moment correlation coefficient's assumptions had not met.

RESULTS

Demographic and anthropometric measurements for the Asian Indians and Caucasians are presented in **Table 13**. A sample of 32 males was included in the analysis of the study. Sixteen subjects self-identified themselves as Asian Indian and 16 males self-identified themselves as Caucasians. There was no significant difference between the two groups in age. The median age was 25 years for Asian Indians and 28 years for Caucasians, U=123, p = 0.85. For the anthropometric measurements, Asian Indians have

significantly lower body weight, U= 59, p = 0.01, and short stature, U = 44.5, p < 0 .01, compared to Caucasians. However, there was no difference in the BMI between groups U= 100, p 0.29. Out of the 16 Asian Indians, there were 6 participants in the normal weight category, 7 participants in the overweight category and 3 participants in obese category according to the BMI cut off for Asian population[29]. Out of the 16 Caucasians, there were 7 participants in the normal weight category, 5 participants in the overweight category, and 4 participants obese category according to the international BMI cut off established by World Health Organization [29]. There were no differences in the percentage of normal weight, overweight and obese between the two ethnic groups $\chi 2$ (2, n=32) =0.55, p = 0.76). Asian Indians had a lower average mean waist circumference (34.05 ± 5.62 inches) compared to Caucasians (36.43 ± 4.46 inches) (t (30) = -1.33, p =0.20. There was no difference in the proportion of Asian Indians and Caucasians with central adiposity according to the waist circumference measurements $\chi 2$ (1, n=32) =1.39, p = 0.24.

TABLE 13 Demographics Characteristics and Anthropometrics Measurements by Ethnic Groups [a -g]

Variables	Asian Indians	Caucasians	P value
Age (years)	25.00 (9.0)	28.00 (10.0)	0.85 [a]
Weight (lb.)	157.88 (38.56)	185.88 (60.63)	**0.01*** [a]
Height (inches)	67.13 (4.19)	72.13 (4.10)	**<0.01*** [a]
BMI (kg/m^2)	24.91 (4.63)	25.42 (5.02)	0.29 [a]
Normal Weight Category n (%) [b]	6.0 (37.5)	7.0 (43.8)	0.76[e]
Overweight Category n (%)[c]	7.0 (43.8)	5.0 (31.3)	
Obese Category n (%)[d]	3.0 (18.8)	4.0 (25)	
Waist Circumference (inches)	34.05 ± 5.62	36.43 ± 4.46	0.20[f]
Central Adiposity n (%)[g]	6.0 (37.5)	3.0 (18.8)	0.24[e]

Values are Mean ± SD or Median (interquartile range)
* $p \leq 0.05$ indicates a significant difference between groups
[a] Mann Whitney U test
[b] Normal weight category of BMI for Asian Indians ranges between 18.5 – 22.9 kg/m^2 and for Caucasians ranges between 18.5 – 24.9 kg/m^2
[c] Overweight category of BMI for Asian Indians ranges between 23.0 – 27.4 kg/m^2 and for Caucasians ranges between 25.0 – 29.9 kg/m^2
[d] Obese category for BMI for Asian Indians ranges between 27.5 – 35 kg/m^2 and for Caucasians ranges between 30.0 – 35 kg/m^2
[e] Chi-square contingency table test
[f] Independent Sample T-test.
[g] Central adiposity is defined with waist circumference ≥ 35 inches in Asian Indians and ≥ 40 inches in Caucasians
Abbreviation: lb; pound, kg; kilogram, m^2; meters squared.

Bone Outcomes

Areal bone mineral density, bone mineral content (BMC) and BMAD for Asian Indians and Caucasians are presented in **Table 14**. The $_a$BMD at the lumbar spine,

femoral neck and 33% radius of the non-dominant hand were slightly lower in Asian Indian males compared to Caucasians; however, none of them were significant at p <0.05. A trend p-value toward significance was found in the $_{a}$BMD at the whole body. Means $_{a}$BMD at the whole body were 1.23 ± 0.13 gm/cm^2 in Asian Indians and 1.34 ± 0.12 gm/cm^2 in Caucasians t (30) = -1.88, p 0.07. BMC at all measured sites were significantly lower in Asian Indians than Caucasians, p < 0.05 except for the femoral neck. There was a trend toward significance in the BMC at the femoral neck t (30) = -1.92, p 0.07. None of the BMAD at any sites measured were significantly different between the two groups, p >0.05.

TABLE 14
Areal Bone Mineral Density, Bone Mineral Content, and Bone Mineral Apparent Density by Ethnic Groups

Variables	Asian Indians	Caucasians	P value
$_{a}$BMD (gm/cm^2)			
Whole Body	1.26 ± 0.13	1.34 ± 0.12	0.07
Lumbar Spine (L $_{2}$- L $_{4}$)	1.26 ± 0.18	1.35 ± 0.18	0.15
Femoral Neck	1.13 ± 0.15	1.16 ± 0.18	0.58
33% Radius	0.99 ± 0.08	1.00 ± 0.09	0.75
BMC (gm)			
Whole Body	2878.75 ± 375.90	3419.69 ± 512.62	**<0.01** *
Lumbar Spine (L $_{2}$- L $_{4}$)	56.33 ± 10.21	71.21 ± 16.41	**<0.01***
Femoral Neck	5.91 ± 0.83	6.63 ± 1.27	0.07
33% Radius	2.53 ± 0.36	2.81 ± 0.40	**0.04***
BMAD (gm/cm^3)			

Whole Body	0.72 ± 0.07	0.73 ± 0.06	0.81
Lumbar Spine (L_2- L_4)	0.73 ± 0.11	0.74 ± 0.08	0.80
Femoral Neck	0.65 ± 0.09	0.63 ± 0.08	0.51
33% Radius	0.57 ± 0.05	0.55 ± 0.05	0.13

All values are Mean ± SD
* $p \leq 0.05$ indicates a significant difference between groups
Abbreviation: $_a$BMD; areal bone mineral density, gm; grams, cm^2; centimeters squared, BMC; bone mineral content, BMAD; bone mineral apparent density, cm^3; centimeters cubic.

Body Composition Outcomes

The differences in body composition variables between Asian Indians and Caucasians are presented in **Table 15**. There were no significant differences between Asian Indians and Caucasians for total fat mass, android fat mass, gynoid fat mass, visceral adipose tissue and subcutaneous adipose tissue, $p > 0.05$. A significant difference was found in the lean mass. Asian Indians have lower lean mass (122.43 ± 18.88 lb.) compared to Caucasians (137.89 ± 19.14 lb.) ($t(30) = -2.31$, $p = 0.03$.

TABLE 15
Body Composition Outcomes by Ethnic Groups [a-c]

Variables	Asian Indians	Caucasians	P value
Lean Mass (lb)	122 .43 ± 18.88	137.89 ± 19.14	**0.03***[a]
Fat Mass (lb)	46.75 (19.18)	49.60 (18.98)	0.62 [b]
Android Fat Mass (lb)	3.80 (2.55)	4.00 (2.55)	0.46 [b]
Gynoid Fat Mass (lb)	7.60 (2.95)	8.20 (3.58)	0.49 [b]
Android Fat Mass /Gynoid Fat Mass Ratio	0.49 ± 0.11	0.50 ± 0.11	0.83 [a]
Visceral Adipose Tissue (lb)[c]	0.89 ± 0.65	1.68 ± 1.03	0.14 [a]
Subcutaneous Adipose Tissue (lb)[c]	1.88 (2.43)	2.20 (1.85)	0.42 [b]

Values are Mean ± SD or Median (interquartile range)
* p <0.05 indicates a significant difference between groups
[a] Independent Sample T-test
[b] Mann Whitney U test
[c] Variables were measured only for 12 participants out of the 32 participants
Abbreviation: lb; pound

Dietary Intake Outcomes

The differences in nutrient intake between Asian Indians and Caucasians are presented in **Table 16**. The caloric intake per day was similar between Asian Indians and Caucasians t (30) = 0.14, p =0.89. The calories consumed per weight was slightly higher in Asian Indians (27.98 ± 10.85 kcal/kg) than Caucasians (23.61 ± 6.60 kcal/kg), but it was not significant p= 0.18. There were no significant differences between groups for protein, carbohydrates, fat, calcium, magnesium and vitamin D, p >0.05. Thirteen (81.3%) Asians Indians met calcium's RDA by the Indian Council of Medical Research

while only 9 (56.3%) Caucasians met calcium's RDA by Institute of Medicine, $\chi 2$ (0, n=32) =2.33, p = 0.13.

TABLE 16
Dietary Intakes by Ethnic Groups [a-d]

Variables	Asian Indians	Caucasians	P value
Calories (kcal)	2169.5 ± 947.7	2127.9 ± 694.8	0.89[a]
Calories/ Weight (kcal/kg)	28.0 ± 10.9	23.6 ± 6.6	0.18[a]
Carbohydrates (gm)	249.6 ± 139.9	235.4 ± 82.7	0.73[a]
Protein (gm)	111. 5 ± 59.9	98.4 ± 44.9	0.49[a]
Protein/ weight (gm/kg)	1.3 (1.4)	1.0 (0.7)	0.31[b]
Fat (gm)	79.5 ± 36.7	75.9 ± 41.4	0.8[a]
Calcium (mg)	1102. 6 (722.3)	937.2 (623.9)	0.16[b]
Meeting RDA n (%)[c]	13 (81.3)	9 (56.3)	0.13[d]
Vitamin D (mcg)	1.2 (3.5)	0.6 (3.5)	0.81[b]
Magnesium (mg)	255.3 (238.2)	243.6 (199.7)	0.88[b]

Values are presented as Mean ± SD or Median (interquartile range)
* $p \leq 0.05$ indicates a significant difference between groups
[a] Independent Sample T-test
[b] Mann Whitney U test.
[c] Calcium RDA for Asian Indians is 600 mg /day and 1000 mg/day for Caucasians
[d] Chi-square contingency table test
Abbreviation: Kcal; kilocalories, Kg; kilograms, gm; grams, mg; milligrams, mcg; micrograms.

Correlation between body composition and Areal Bone Mineral Density

The correlation between body composition and $_{a}$BMD are provided in **Table 17**. A Spearman's rank correlation between lean mass and $_{a}$BMD at the lumbar spine was statistically significant in the overall sample (r= 0.44, p =0.01). However, within each ethnic group, the positive relationship between lean mass and $_{a}$BMD at the lumbar spine was not evident, p >0.05. Regarding the association between lean mass and $_{a}$BMD at the femoral neck, no associations were found in the overall sample, p= 0.097, or Asian Indians, p = 0.47. However, a positive association was revealed in Caucasians (r= 0.53, p = 0.03) between lean mass and $_{a}$BMD at the femoral neck.

Android fat mass had negative associations, though insignificant, with $_{a}$BMD at the lumbar spine and femoral neck at the overall sample. Caucasians maintained the negative, insignificant association between android fat and $_{a}$BMD at the lumbar spine (r= - 0.26, p =0.33) and $_{a}$BMD at the femoral neck (r= -0.05, p= 0.85) when they were analyzed as a group. Asian Indians showed a site-specific relationship between android fat and $_{a}$BMD. A negative association was shown between android fat and $_{a}$BMD at the femoral neck (r= -0.05, p= 0.85), while insignificant positive association with $_{a}$BMD at the lumbar spine (r= 0.12, p= 0.67).

Gynoid fat was not associated with any of $_{a}$BMD sites in the overall sample or within each group. Interestingly, gynoid fat has a positive association with $_{a}$BMD at the lumbar spine (r=0.06) while the negative association with $_{a}$BMD at the femoral neck (r= -0.03) in the overall sample. None of the association was significant at the alpha level of 0.05. Asian Indians showed a similar site-specific association between gynoid fat and

$_{a}$BMD. On the other hand, Caucasians showed negative, insignificant association between gynoid fat and $_{a}$BMD at the lumbar spine and femoral neck.

The comparison of the correlations between body composition and $_{a}$BMD at the lumbar spine and femoral neck was listed in **Table 18**. Fisher z transformation was performed to compare the relationships between each variable of the body composition with $_{a}$BMD at the lumbar spine and femoral neck between Asian Indians and Caucasians. A z score of more than 1.96 or less than -1.96 shows a significant difference in the correlation between the two groups at the alpha level of 0.05. No significant difference was found in the correlation between body composition variables and $_{a}$BMD at the lumbar spine and femoral neck between Asian Indians and Caucasians.

TABLE 17
Correlation Coefficients Between Body Composition Variables and Areal Bone Mineral Density [a-b]

Variables	**Groups**	**Lean Mass** (lb)		**Android Fat Mass** (lb)		**Gynoid Fat Mass** (lb)	
		r	**P value**	**r**	**P value**	**r**	**P value**
Lumbar Spine (L_2- L_4)	Total Sample N=32	0.44 [a]	**0.01***	-0.02 [a]	0.90	0.06 [a]	0.77
	Asian Indians n=16	0.35[b]	0.19	0.12 [a]	0.67	0.15 [a]	0.57
	Caucasians n=16	0.44 [b]	0.09	-0.26 [a]	0.33	-0.16 [a]	0.57
Femoral Neck	Total Sample N=32	0.30 [a]	0.10	-0.19 [a]	0.92	-0.03 [a]	0.89
	Asian Indians n=16	0.20 [b]	0.47	-0.05 [a]	0.85	-0.03 [a]	0.92
	Caucasians n=16	0.53 [b]	**0.03***	-0.05 [a]	0.85	-0.11 [a]	0.70

* p <0.05 indicates a significant correlation
[a] Spearman's rank correlation coefficient
[b] Pearson product-moment correlation coefficient
Abbreviation: $_a$BMD; areal bone mineral density, gm; grams, cm^2; centimeter, lb.; pound, r; correlation coefficient

TABLE 18
Comparing the Strength of the Correction between Body Composition Variables and Areal Bone Mineral Density by Ethnic groups

Variables		**Asian Indians**	**Caucasians**	**Z score**	**P value**
$_{a}$BMD (gm/cm^2)	Body Composition (lb)	**r**	**r**		
Lumbar Spine (L_2 - L_4)	Lean Mass	0.35	0.44	-0.28	0.78
	Android Fat	0.12	-0.26	0.98	0.33
	Gynoid fat	0.15	-0.16	0.79	0.43
Femoral Neck	Lean Mass	0.20	0.53	-1.01	0.31
	Android Fat	-0.05	-0.05	0.00	1.00
	Gynoid fat	-0.03	-0.11	0.20	0.84

* $p \leq 0.05$ indicates a significant difference in the correlations between Asian Indians and Caucasians
Abbreviation: $_{a}$BMD; areal bone mineral density, gm; grams, cm^2; centimeter, Ib.; pound, r; correlation coefficient

DISCUSSION

Our findings indicate that $_{a}$BMD and BMAD at all measured sites were similar, between Asian Indians and Caucasians. However, BMC was lower in Asian Indians compared to Caucasians at the four skeletal sites. Lean mass was the only significant difference between groups in term of body composition. Lean mass was lower in Asian Indians compared to age and BMI matched Caucasians. In addition, there was no difference in daily calcium and magnesium intakes between Asian Indians and Caucasians.

Bone Outcomes Differences

Other researchers have observed similar results in findings no difference between immigrant Asian Indians and Caucasians in their $_{a}$BMD [15,30-33]. Interestingly, the mean $_{a}$BMD at all skeletal sites observed in our study was higher than the values reported from normative data of $_{a}$BMD for Asian Indian living in India [6,7,34]. On the contrary to the findings reported by the present study, previous studies found that Asian Indians have lower $_{a}$BMD compared to Caucasians[6,7,34]. The variance of the studies protocol could be the reason for such difference in findings. Some of these investigations used National Health and Nutrition Examination Survey (NHANES) III data to compare the difference in $_{a}$BMD between Asian Indians and Americans [6]. In fact, NHANES III data was composed of three different ethnic groups, which were non-Hispanic White, non-Hispanic black and Mexican Americans. As a result, the use of NHANES III without excluding other ethnic groups artificially magnifies difference in $_{a}$BMD between Caucasians and Asian Indians. A similar approach was taken by Marwaha et al. of comparing the $_{a}$BMD between Asian Indians and US reference data provided by DXA manefauactures[35]. Shivane et al. used reference data for Caucasians only and found Asian Indians have lower $_{a}$BMD than Caucasians[8]. The failure to detect such difference by our study could be attributed to the difference in dietary protein and calcium intakes by Asian Indians seen in the present study, which is higher than what has been reported before for Asian Indians living in India [8,36]. The mean protein intake reported in the study was close to the mean protein intake reported by other researchers from military personnel [35,37], who failed to find a difference in $_{a}$BMD between Asian Indians and Caucasians. Higher

protein intake enhances bone density by being a structural component of the bone and enhancing the concentration of Insulin-like Growth Factor 1 (IGF-1) [38]. Our data revealed the similarity in $_{a}$BMD between young Asian Indian immigrants and Caucasians.

The significant difference in BMC at all skeletal sites between the two ethnic groups noticed in this study agrees with previous studies[15-17]. Since both $_{a}$BMD and BMC are influenced by the skeletal size[39], both outcomes might be artificially low in Asian Indians than Caucasians if the former group are shorter. As a result, in the current study, the approach of eliminating the dependence of $_{a}$BMD on frame size was by using BMAD. Our data revealed the lack of significant difference in the BMAD between the Asian Indians and Caucasians in all measured skeletal sites, which supported by other studies conducted in both sexes, males[15,30,40] and females[23,32,41].

Dietary Intakes

Our data showed that Asian Indians tended to consume more calcium per day compared to Caucasians. Despite the lack of statistical difference in mean calcium intake between Asian Indians and Caucasians, most of Asian Indians met Calcium's RDA but Caucasians did not. However, the percentage of participants meeting Calcium's RDA did not differ between the two ethnic groups. The calcium intake reported from the *What We Eat In America, NHANES* 2009-2011 showed that the average intake of males met the RDA[42], but the current study found that Caucasians consumed less calcium per day and did not meet their calcium's RDA of 1000 mg/day. Interestingly, in the current study, the calcium intake by Asian Indians was similar to what has been reported on Asian Indians for military personals[37] and slightly higher than reported in Asian Indian in the United

States[36], but dramatically higher than the general Asian Indian population[8]. Shatrugna et al. found lower intake of calcium by Asian Indians women from low socioeconomic status[43]. The difference in the findings could be due to the sex difference between the two studies' sample. In general, men consume more calories and thus, micronutrients than women do. The economic status and the degree of urbanization would play a role as well. The daily intake of calcium among healthy Asian Indian males age 20 to 29 years from upper socioeconomic status was 1194 ± 513.1 mg/day[6]. As evidence of the effect of urbanization on a diet, Asian Indian residents in urban areas consumed more calcium than Asian Indian resident in rural areas because of the obvious change in macronutrient partitioning [44]. In Asian Indian community, the higher the socioeconomic status is, the higher consumption of calories and the education level are [45]. These could apply to the Asian Indians in the United States as well and justify why there was higher consumption of protein and calcium among Asian Indians. In our sample, both Asian Indians and Caucasians failed to meet the magnesium's RDA. The finding for Asian Indians was similar to the finding by Shah et al. They found that the intake of magnesium was 240 ± 129 mg day in young healthy Asian Indian in the United States [36]. Data from NHANES for 1999-2000[46] and 2001–2014[47] are congruent with our findings of magnesium intake by Caucasians. We found no difference in calcium or magnesium intakes between young Asian Indians and Caucasians.

Body Composition

The findings from the current study confirmed the significant difference in lean mass between Asian Indians and Caucasians seen by others [16,48-50]. With regard to the fat

mass difference, previous investigations have shown that Asian Indians have higher abdominal fat mass compared to Caucasians despite similar or lower circumference of the former group[51]. The current study, however, found no differences in android and gynoid fat, and visceral and subcutaneous adipose tissue. As a result, the findings from current study disagreed with ample of evidence which found that Asian Indians have higher total fat mass and regional fat mass compared to Caucasians. Nevertheless, there were some studies supporting the absence of difference in subcutaneous adipose tissue and visceral adipose tissue measured by the gold standard technique, Magnetic Resonance Imaging, between Asian Indians and Europeans [48]. The current study found that Caucasians have insignificant higher visceral adipose tissue compared to Asian Indians which was seen by others [48,50]. We had the measurements of visceral adipose tissue and subcutaneous adipose tissue for 12 participants only. Among this small subgroup, the median waist circumference was higher in Caucasians than Asian Indians, and the trend p-value was close to significance, although the difference was not significant ($p= 0.078$). The lack of difference in visceral and subcutaneous adipose tissue could be because the mean waist circumference for Asian Indians in the current study was slightly lower compared to what had reported by others [48,52] and their Caucasian counterparts in the present study. In addition, the Caucasian males recruited in this study had slightly higher BMI and age compared to Asian Indians. The difference in the fat distribution between Asian Indians and Caucasians may be become more evident with higher BMI[48] and in older age. Another reason which explains the lack of difference in fat mass in the present study is that we measured the fat mass, gynoid and android mass as a mass without adjusting

these values for body weight. Previous study has shown that the fat mass itself did differ between Asian Indians and Europeans, but after adjustment for body height and weight, it became significantly higher in Asian Indians [17]. Our data showed that Asian Indians had lower lean mass than Caucasians, but no difference in android or gynoid mass was detected.

The Association Between Body Composition and Areal Bone Mineral Density

It was found in the present study that positive associations between lean mass with $_a$BMD at the lumbar spine in the overall sample and $_a$BMD at the femoral neck in Caucasian participants only. The correlation coefficients were positive but not significant for Asian Indians. Country to our findings on Asian Indians, previous investigators have shown a significant positive effect of lean mass on $_a$BMD at the lumbar spine and femoral neck in a large sample size [53]. Similar findings were observed by other researchers in different ethnic groups[11,12,54-56]. The reason behind the lack of any significant positive correlation with $_a$BMD is the low lean mass in Asian Indians. In like manner, meta-analysis has shown that the effect of lean mass on $_a$BMD is much stronger in men than women because of the higher lean mass in the former group [57]. Furthermore, the positive effect of lean mass on $_a$BMD is diminished by sedentary lifestyle[11]. Previous works have found Asian Indians have lower levels of physical activity compared to Caucasians[30,58]. The level of physical activity might be another reason for detecting the positive association between lean mass and $_a$BMD with small sample size in Caucasians but not in Asian Indians.

The lack of association between fat mass and $_a$BMD was the results of this study. Some researchers found positive association[54,56,59,60], while other found negative association[11,12,53,54,61] or lack of the association at all[11,54,55,59]. The inconsistency in findings is due to the complex relationship between fat mass and $_a$BMD. The association between fat mass and $_a$BMD is influenced by a sex[39], age[11], ethnicity, skeletal sites and the stage of bone mass-bone maintenance or bone loss[11]. Cheng et al. found men aged 20 to 50 years did not benefit from the fat mass, while older men did[11]. Also, Bogl et al. claimed that the presence of multiple confounding variables in the association between fat mass and $_a$BMD such as diet, socioeconomic status, genetic and environmental factors and physical activity make the association between fat mass and $_a$BMD inconsistent [62]. At the cellular level, with the recognition of fat mass as an active endocrine organ, fat mass secretes active metabolites and hormones involved in the bone metabolism whether favorably such as 17-estradiol or negatively such as inflammatory cytokines[63,64]. Furthermore, osteoblast and adipocyte cells are originated from the same progenitor in the bone marrow, which is the mesenchymal stem cell [63-65]. The differentiation of mesenchymal stem to either cell is influenced by some cytokines secreted by adipocytes [66].

The current study examined the association between regional fat distribution and $_a$BMD, which was investigated by few researchers. However, most of the studies focused on the relationship between total fat mass and $_a$BMD. We used the advantages of DXA to measure regional fat distribution to gauge visceral adipose tissue by measuring android fat and subcutaneous adipose tissue by measuring gynoid fat. Our data revealed mixed

finding between gynoid fat and $_{a}$BMD, but none of them were significant. Previous investigations showed negative [67,68] positive [69,70] or lack of association[71] between subcutaneous adipose tissue and $_{a}$BMD. Most of the previous works showed a negative relationship between visceral adipose tissue and $_{a}$BMD [67-71]. The findings seen in the current study partially agreed with previous findings; however, none of the associations were significant. The lack of significance was due to the use of an indirect approach to measuring visceral adipose tissue. The android fat measured in this study contained both visceral adipose tissue and subcutaneous adipose tissue. The visceral and subcutaneous adipose tissue are distinct fat compartments. They differ in the secretion of hormones and peptides, function, adipocytes size, blood vascularity and innervations and risk of developing chronic diseases[72]. Our data showed no association between android or gynoid fat with $_{a}$BMD in Asian Indians or Caucasians.

Despite the lack of significant difference in the correlation between body composition with $_{a}$BMD in Asian Indians and Caucasians, the differences in the direction of the association between android and gynoid fat with $_{a}$BMD were noticed between Asian Indians and Caucasians raise a flag to consider ethnic-specific understanding of the association between body composition and $_{a}$BMD.

The current study has several strengths. First, a unique perspective was used by comparing bone outcomes, dietary intake and body composition in healthy young Asian Indian immigrants in the United States with Caucasians matched for age and BMI in a single study. Second, the two groups were matched based on age and BMI to control for those confounding variables while comparing the difference in bone and body

composition and testing the correlations between body composition and $_{a}$BMD. That was done because both variables have their influences on the primary outcomes of the current study. Additionally, the lean mass was assessed independently from the bone mass. In other words, we measured bone-free lean mass which a limitation in some of the previous investigations. Also, the calcium and magnesium intakes were assessed using food frequency questionnaires to capture the intake of these nutrients over six months, which matches the rate of bone mineral density turnover.

The study has some limitations. Firstly, the physical activity was not measured using an objective and valid assessment tool. The assessment of physical activity in the study was limited to a self-reported level of physical activity in the 24 hours preceding the day of collecting all measurements. Physical activity positively influences both lean mass and bone density, especially during bone accrual stage. As a result, physical activity could be confounding variables for the associations between body composition and $_{a}$BMD. Additionally, the study did not investigate inflammatory cytokines and bone turnover markers, which could add a new dimension in understating some aspect of bone quality. Finally, we did not distinguish between first and second generation Asian Indian immigrants. This may have been important because second-generation immigrant might undergo through the acclimatization to food, environmental risk factors, health awareness and access to health care which is not could be not common for first-generation immigrants. Finally, the study had a small sample, which may have limited certain variables from reaching statistical significance. However, the current experimental

research aimed to set the stage for future studies in understanding the impact of body compositions on $_{a}$BMD of Asian Indian and Caucasian men.

Conclusion

Our data indicates that there were no differences in aBMD, calcium and magnesium intakes between healthy young Caucasians and Asian Indians in the United States. However, Asian Indians have markedly lower lean mass compared to Caucasians. Since the scope of this study was limited to examine the difference in bone density using $_{a}$BMD, the study highlights the need for further research to examine the variability in bone turnover between immigrant Asian Indians and Caucasians to see if the findings from biochemical outcomes and bone density are similar.

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Appendix 1

Recruiting Volunteers for a Research Study

Bone and Metabolic syndrome study

Research Objective

To determine the relationship between bone health and metabolic syndrome outcomes

Who is eligible?

You should be a South Asian or Caucasian male between ages 20-50 years.

You should have a BMI between 23kg/m^2 and 35 kg/m^2.

What is involved?

We will measure your bone density and body composition with a DXA.

We will also measure your vitamin D status and metabolic profile.

Study Visits and Compensation

1 study visit, $30 compensation. Free body composition test!

If you are interested in participating in this study, please contact:

Dr. Deeptha Sukumar May Cheung

Email: **projectvitd@drexel.edu** Phone: (267)359-5854

Room 317, 1601 Cherry Street, Philadelphia, PA 19102

This research is conducted by a researcher who is a member of Drexel University

Appendix 2

Drexel University
Telephone Screening Form

Name of Screener:_____ Date:_____

The **purpose** of this screening interview is to see if you meet the criteria for taking part in our **research** study of the influence of ethnicity on bone mineral density (BMD), body composition and metabolic syndrome biomarkers in South Asian Indian men compared to Caucasian men. This interview will take approximately 20 minutes. I am going to go through a list of questions. You may choose not to answer these questions. You also may choose to stop participating in this interview at any time; if you want to stop, please tell me.

Time

This interview will take about 20 minutes

Confidentiality

Information about you that you give me during this interview will be kept as **confidential** as possible as required by law. It is possible that the Food and Drug Administration, and other federal and state authorities, may inspect this record.

Freedom to withdrawal

You can **choose** if you want or do not want to take part in this research screening procedure – it is up to you. If you refuse to answer the questions or stop answering them at any time, there will be **no penalty**, and you will not lose any benefits to which you otherwise would be entitled.

Risks or Discomforts

The **risk** to taking part in this interview is very small. The screening interview is not designed to ask you for sensitive personal information, but it is possible that some people may feel **uncomfortable** answering these questions with a person they do not know. If you qualify to take part in the study and are *interested* in taking part, then I will record your name and information; this will be kept confidential, but there is a small risk that people outside of the research team *(or University/Hospital - state relevant unit)* could learn this information. If you are *not interested* in the study, then I will destroy the personal information you give me.

Benefits

The benefit to you of taking part in this interview is that you will find out whether you can take part in the study of determinants of bone mineral density and metabolic syndrome in South Asian Indian Men.

Procedures

This study involves a one-time visit that will last no longer than 2 hours. You will be asked to provide a blood sample and a urine sample during this visit. You will also be asked to fill out study questionnaires. We will assess your dietary intake of these nutrients along with other nutrients using these questionnaires. You will also receive a DXA scan.

Alternatives

You will not be paid for answering questions in this interview since it is only to see whether you qualify to take part in the study.

Contact

If you have any questions, concerns, or complaints about this interview, contact [*Dr Deeptha Sukumar, Phone 215-359 5854*. If you want to talk to someone separate from the research team about a concern or complaint or your rights as a possible research subject, please contact Human Research Protection at 215-255-7857.

Subject Information:
Last Name:_____First Name:_____
Home Phone:_____Work Phone:_____
Cell Phone:_____Email:_____

Begin Telephone Screen:
"Hi, my name is . I am returning your call regarding the Research study. Thank you for your interest. To begin, let me give you a little back ground about our study. We are interested in determining how ethnicity influences bone mineral density, body composition, and metabolic syndrome related biomarkers in South Asian Indian men."
" This one-time visit will consist of blood/ urine tests and a DXA scan. You will receive a $30 compensation for this visit. If you have a couple of minutes right now, I would like to ask you a few questions to determine if you are eligible to participate in the study."
How did you hear about this study_____?
Note: If you are uncertain about any eligibility question, tell the person you will check and call her back.
If at any point the person is no longer eligible, please say something that sounds like the following:
I am sorry but you do not fall within the inclusion criteria, I must end the interview at this time. Thank you for your interest in this study."
If Eligible Continue Here:

60 Inches = 5'
65 Inches = 5'5"
70 Inches = 5'10"
72 Inches = 6'

1. "What is your ethnicity? _____
2. *"What is your age?"*_____years
3. *"What is your current weight?"*_____pounds
4. *"Have you gained or lost weight in past few months?"* _____
5. *"How tall are you?"*_____Inches **(5 ft = 60 inches)**

Please circle and write the subject's BMI on the chart on the next page.
Continue questionnaire if BMI is over 23kg/m2.
BMI_____
5. *"Do you have any medical problems?"*
USE LIST BELOW TO ASK ABOUT THIS. Exclude subject if he has any of the following conditions or is taking medications for the any of the following conditions:

Condition	Yes	No
Diabetes treated with medication		
Cancer or cancer therapy within the past year		
Kidney Disease or stones within the past 5 years		
Immune Disease or on steroids to suppress immune function		
Thyroid Disorders only if unmanageable; allow if their condition is stable		
Liver Disease		
Heart Attack or Stroke in the past 6 months		
Arthritis only if on prednisone (steroid)		

6 "Are you taking any medications?"
Yes □ No □
If Yes, please list:_____
7 *"Have you stopped taking any medications in the past year?"*
Yes □ No □ If Yes, please list:_____
"How long did you take them for?" _____

8 *Do you take any osteoporosis medication* (HRT, Fosomax, Actonel, Evista, Forteo, etc.)?

9 "Have you experienced a fracture after the age of 45?" Yes □ No □
If Yes, then "Was *it traumatic – and where was it?"* _____
(We are mostly worried about serious fractures)

10 "Are you taking any vitamin, mineral or herbal nutritional supplements?"
Yes □ No □
If Yes, "*Would you be willing to alter their regimen for the study?"***:**_____
(Exclude if not willing. Be sure to assure them they will be taking a Vit/Min regimen recommended by us if they are eligible to participate in the study)

11 "Do you have any food allergies (lactose intolerance, allergic to wheat, etc)?"
Yes □ No □
If Yes, please list:_____

Eligibility checklist:
- □ **BMI within range (23 – 35 kg/m2)**
- □ **SAI or Caucasian male**
- □ **Age 30-50 years**
- □ **No major medical problems and medications**
- □ **Not on Hormone Replacement Therapy (HRT) or osteoporosis meds (past 6 months)**

Eligible Participant
Based on the information you gave me, it looks like you are eligible for this study. At this point, you have three choices. (1) I can take down your contact information and have our staff contact you to set up an appointment; or (2) I can give you the number to call to set up an appointment yourself; or (3) if you are not interested in learning more about the study, you should say that and I will not keep the information collected in this interview.

_____ OK TO CONTACT *(collect contact info)*
_____ SUBJECT TO CONTACT *(give contact info)*
_____ NOT INTERESTED → **(*destroy <u>all</u> information collected*)**
_____ NOT INTERESTED <u>IN THIS STUDY, BUT INTERESTED IN OTHER STUDIES</u> → I would like to offer you some phone numbers and referral information for other programs in the area that might be better able to meet your needs at this time. Would you like us to either call you back or mail that kind of information to you?
_____ CALL BACK → (Phone #: _____)
_____ MAIL ADDRESS → _____)
Thank you for your time.

Preferred Contact Method (check one):
Home Phone □ **Work Phone** □ **Cell Phone** □ **Email** □

□ **Check if contact successful**

If unsuccessful (enter date and time of call)
1st attempt on _____
2nd attempt on _____
3rd attempt on _____

□ Successful Contact on_____
Notes _____
Ineligible Participant: Based on the information you gave me, you are not eligible for this study. Thank you for your time

Appendix 3

Drexel University

Consent to Take Part

In a Research Study

1. Title of research study : Determinants of Bone Mineral Density and Metabolic Syndrome in South Asian Indian Men

2. Researcher: Deeptha Sukumar, PhD

3. Why you are being invited to take part in a research study

We invite you to take part in a research study because you meet the study criteria of being a South Asian Indian (SAI) or Caucasian male of ages within 20-50 years and have a Body Mass Index between 23 kg/m^2 and 35 kg/m^2.

4. What you should know about a research study

Someone will explain this research study to you.
Whether or not you take part is up to you.
You can choose not to take part.
You can agree to take part now and change your mind later.
If you decide to not be a part of this research no one will hold it against you.
Feel free to ask all the questions you want before you decide.

5. Who can you talk to about this research study?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at ***[267 359 5854 or deeptha.sukumar@drexel.edu]***

This research has been reviewed and approved by an Institutional Review Board (IRB). An IRB reviews research projects so that steps are taken to protect the rights and welfare of human subjects taking part in research. You may talk to them at (215) 255-7857 or email HRPP@drexel.edu for any of the following:

Your questions, concerns, or complaints are not being answered by the research team.
You cannot reach the research team.
You want to talk to someone besides the research team.
You have questions about your rights as a research subject.
You want to get information or provide input about this research.

6. Why are we doing this research?

This study is designed to investigate the influence of ethnicity on body composition and bone density. The SAI population has both a lower bone density and a greater risk of metabolic syndrome (MetS). MetS is a cluster of risk factors that increases the risk of cardiovascular disease and type 2 diabetes. The incidence of low bone mineral density is also higher in SAI compared to the Caucasian population. This study will examine whether or not these metabolic and biochemical markers (Indicators of bone building and breaking in the blood and urine, Lipids and other proteins) explain both low BMD and MetS in SAI men.

7. How long will the research last?

This study consists of a one-time visit no longer than 2 hours.

8. How many people will be studied?

We expect about 60 people (30 SAIs and 30 Caucasians) will be in this research study.

9. What happens if I say yes, I want to be in this research?

If you are willing to participate in this study, you will be required to come to the research facility at 3rd floor- Room 317, 1601 Cherry street, Phildelphia, PA for one visit.

Parcipants will expect the following during their visit:

- *This visit will last for about 2 hours*
- *Blood draw- Four 5 ml tubes of blood will be collected via venipuncture*
- *Urine sample collection: You will be asked to void a small amount of urine into the cup that is provided to you.*
- *Measurement of height, weight, blood pressure, waist circumference*
- *Completion of study questionnaires- This will include food diaries, medical history questionnaires and food frequency questionnaires. All these can be completed in less than 20 minutes.*
- *DXA scan : This scan will enable us to measure your bone mineral density and body composition. It also exposes you to a small amount of radiation which is typically less than a day's exposure to natural radiation.*

10. What are my responsibilities if I take part in this research?

If you take part in this research, it is very important that you follow your physician's or researcher's instructions.

11. What happens if I do not want to be in this research?

You may decide not to take part in the research and it will not be held against you.

12. What happens if I say yes, but I change my mind later?

You agree to take part in the research now and stop at any time it will not be held against you.

If you stop being in the research, already collected data may not be removed from the study database. You will be asked whether the researcher can collect data from your routine medical care. If you agree, this data will be handled the same as research data.

13. Is there any way being in this study could be bad for me?

There should be no discomforts in this study other than the blood drawing which can result in a temporary slight discoloration in the skin surrounding it. The DXA scan will expose you to very small amounts of radiation. The risks from this exposure if they exist at all are minimal.
You and your insurance company will be charged for the health care services that you would ordinarily be responsible to pay. In some cases, insurance will not pay for services ordinarily covered because these services were performed in a research study. You should check with your insurance to see what services will be covered by your insurance and what you will be responsible to pay.

14. Do I have to pay for anything while I am on this study?

There is no cost to you for participating in this study.
You will not be charged for any tests specifically required for this research study, but you or your insurance company will still be billed for tests or procedures that are considered "standard of care" and would have been part of your medical treatment even if you did not participate in this study. These treatment costs include but are not limited to drugs, routine laboratory tests, x-rays,

scans, surgeries, routine medical care, and physician charges. Your health insurance company may not pay for these "standard of care" charges because you are in a research study. If your insurance company
does not pay for costs associated with this research study that are considered "standard of care" for your medical treatment, then you will be billed for these costs. You are responsible for paying for any insurance co-pays and any deductibles due under your insurance policy, and any charges your insurance company does not pay.

15. Will being in this study help me any way?

There are no benefits to you from your taking part in this research.

16. What happens to the information we collect?

Efforts will be made to limit your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

Data or specimens will be retained after the study for future research to examine if certain newly identified biomarkers such as bone turnover markers (these are indicators of bone building and breakdown which can be detected in your blood or urine) are correlated with ethinicity. Data and specimens will be stored in 3711 Market street, Philadelphia, PA . Access to samples beyond completion of the study is limited to only study personnel.

The monitors, auditors, the IRB, the Food and Drug Administration will be granted direct access to your medical records for verification of the research procedures and date. By signing this document you are authorizing this access.

We may publish the results of this research. This includes using data from this research for student research such as a master' s thesis. However, we will keep your name and other identifying information confidential.

17. Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include, failure to follow study protocol, no –show for more than 3 scheduled visits or failure to meet continued study criteria due to illness/medications.

18. What else do I need to know?

This research study is being done by Drexel University. If you become ill or injured during the study, contact Dr Sukumar at 267 359 5854. We will get you medical care. If you need care right away, go to the nearest emergency room or call 9-1-1. Inform all medical emergency staff that you are taking part in this study. If a "research related injury" results from your participation in this research study, medical treatment will be provided. The cost for all your medical treatment will be billed to you or your insurance. "A research related injury means injury caused by products or procedures required by the research, which you would not have experienced if you had not participated in the research"

If you agree to take part in this research study, you will be provided upto $30 compensation for your time and effort.

Federal law provides additional protections of your personal information that are described here.

Authorization to Use and Disclose Protected Health Information

A. Individually Identifiable Health Information That Will Be Collected

The following personal health information about you will be collected and used during the research study and may be given out to others:

Your name, address, telephone number, date of birth;
Personal and family medical history;
Information from laboratory tests, blood and urine tests, x-rays, physical exams and other tests or procedures described in this consent form.
Information learned during telephone calls, surveys, questionnaires and office visits done as part of this research study;

B. Who Will See and Use Your Health Information within Drexel University

The researcher and other authorized individuals involved in the research study at Drexel University will see your health information during and may give out your health information during the research study. These include the researcher and the research staff, the institutional review board and their staff, legal counsel, research office and compliance staff, officers of the organization and other people who need to see the information in order to conduct the research study or make sure it is being done properly. Your health information may be disclosed or transmitted electronically.

C. Who Else May See and Use your Health Information

Other persons and organizations outside of Drexel University may see and use your health information during this research study. These include:

Governmental entities that have the right to see or review your health information, such as The Office for Human Research Protections, and the Food and Drug Administration
Doctors and staff at the hospital where this research study will take place.

If your health information is given to someone not required by law to keep it confidential, then that information may no longer be protected, and may be used or given out without your permission.

D. Why your health information will be used and given out

Your information may also be used to meet the reporting requirements of governmental agencies.

E. If you do not want to give authorization to use your health information

You do not have to give your authorization to use or give out your health information. However, if you do not give authorization, you cannot participate in this research study.

F. How to cancel your authorization

At any time you may cancel your authorization to allow your health information to be used or given out by sending a written notice to Human Research Protection at 1601 Cherry Street, 3 Parkway Bldg., Mail Stop 10-444, Philadelphia, Pennsylvania, 19102. If you leave this research study, no new health information about you will be gathered after you leave. However, information gathered before that date may be used or given out if it is needed for the research study or any follow-up.

***G.* When your authorization ends**

Your authorization to use and give out health information will continue until you withdraw or cancel your authorization.

After the research study is finished, your health information will be maintained in a research database. Drexel University shall not re-use or re-disclose the health information in this database for other purposes unless you give written authorization to do so. However, the Drexel University Institutional Review Board may permit other researchers to see and use your health information under adequate privacy safeguards.

H. Your right to inspect your medical and research records

You will not be able to look at your research records while you are taking part in this research study. Your personal information will be made available in an emergency if doctors need this information to treat you. You can have access to your medical record and any research study information when the study is over. However, the researcher does not have to release research information to you if it is not part of your medical record. Use this paragraph for blinded or other studies where access will be denied.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

DO NOT SIGN THIS FORM AFTER THIS DATE →

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	Form Date

Appendix 4

Please complete the following questions to the best of your ability. If you are unclear what to answer, leave the space blank and we will help with the answer when you are seen at this facility. All answers will be kept in strict confidence and treated as information in your research record.

Name: _____ Today's date: ____ _____
Address: _____ Date of Birth: _____
_____ Race: Caucasian__ Asian__

If you have a preferred contact number, please check

___ Home #: _____ ____ Cell # _____
___ Work # _____ E-mail: _____
Date of Last medical check-up: _____
Height: _____ Weight: _____
Blood Pressure: _____ Waist Circumference: _____

	Yes	No
1. Do you have a medical history of diabetes, hypertension or heart disease?	___	___
2. Do you smoke?	___	___
If yes, how much? _____		
3. Have you smoked in the past?	___	___
If yes, when did you quit? _____		
4. Do you consume alcohol?	___	___
If yes, how many drinks per week? _____		
5. Have you ever been diagnosed with an eating disorder? (binge-eating, anorexia nervosa, laxative abuse? YES_____ NO_____		
If yes please explain_____		

6. Have you had any of the following conditions?:	Y	N
Partial or complete paralysis	___	___
Hyperthyroidism	___	___
Juvenile diabetes	___	___
Hypertension	___	___
Elevated Cholesterol	___	___
Kidney Disease	___	___
Liver Disease	___	___
Rheumatoid Arthritis	___	___
Other Arthritis	___	___
Alcoholism	___	___
Part of stomach removed	___	___

Inflammatory Bowel Disease	___	___
Peptic Ulcers	___	___
Malignant Disease/Cancer	___	___

7. Have you taken any of the following in the last year?

Steroids (prednisone, cortisone, etc)	___	___
Thyroid Medication	___	___
Anticonvulsants	___	___
Diuretics (water pills)	___	___
Gluccocorticoids	___	___
Anticonvulsants	___	___
Blood Thinners	___	___
Antacids	___	___
Antibiotics	___	___
Have you used steroids for more than 1 year?	___	___

8. Do you have any general comments or questions about your past health?
   _____
9. Please list all current medications
   _____
10. Please list medical problems _____
11. Have you recently lost or gained more than 10 lbs? _____
    If yes, please explain: _____

Appendix 5

Subject ID: _____

Weight: _____

Height: _____

Waist circumference: (First measurement) _____

(First measurement) _____

(First measurement) _____

Average of the three readings: _____

Researcher Initial: _____

Date: _____

Appendix 6

MAGNESIUM FOOD FREQUENCY QUESTIONNAIRE

This questionnaire determines your usual eating habits and foods high in magnesium

Record how often you eat each food (daily, weekly or monthly) and write the number in the corresponding column.

		How Often Do You Eat or Drink This Food or Beverage? (Fq)				
Food Source Containing Magnesium		**Magnesium Content**	**# of Times / Day**	**# of Times / Week**	**# of Times / Month**	**TOTAL**
Lentils, raw	1 cup	90				
Collards, cooked, boiled, drained, without salt	1 cup, chopped	40				
Kale, raw	1 cup	8				
Spinach, raw	1 cup	24				
Spinach, frozen, chopped or leaf, cooked, boiled, drained, without salt	½ cup	78				
Nuts, cashew nuts, dry roasted, with salt added	½ cup	178				
Pecans	½ cup, halves	60				
Seeds, pumpkin and squash seeds, whole, roasted, with salt added	1 cup	168				
Seeds, sunflower seed kernels, toasted, with salt added	1 cup	173				
Beans, black, mature seeds, canned, low sodium	1 cup	84				
Chickpeas (garbanzo beans, bengal gram), mature seeds, canned, solids and liquids	1 cup	65				
Salmon, wild, cooked, moist heat	3 oz.	30				
Mackerel, Atlantic, cooked, dry heat	3 oz.	82				
Fish, tuna, light, canned in water, drained solids	3 oz.	20				
Chicken, broiler or fryers, breast, skinless, boneless, meat only, cooked, grilled	3 oz.	29				
Peanut butter, smooth style, with salt	2 tbsp	54				
Peanut butter, chunk style, with salt	2 tbsp	51				
Banana, raw	1 small (6" to 6-7/8" long)	27				

Strawberries, raw	1 cup, whole	19				
Blackberries, raw	1 cup	29				
Raisins, seedless	1 small box (1.5 oz)	14				
Quinoa, cooked	1 cup	118				
Bread, whole-wheat, commercially prepared	1 slice (32g)	24				
Bread, white, commercially prepared (includes soft bread crumbs)	1 slice (25g)	6				
Rice, brown, long-grain, cooked	1 cup	84				
Cereals, oats, regular and quick, not fortified, dry	1 cup	112				
Cereals, QUAKER, QUAKER MultiGrain Oatmeal, dry	1 cup	92				
Yogurt, fruit variety, nonfat	1 container (6 oz)	26				
Milk, whole, 3.25% milkfat, with added vitamin D	8 oz.	24				
Coffee, brewed from grounds, prepared with tap water	8 oz.	7				
Orange juice drink	8 oz.	7				
Candies, SPECIAL DARK Chocolate Bar	1 bar (73g)	23				
Avocado, raw, all commercial varieties	1 cup, cubes	44				

Appendix 7

CALCIUM FOOD FREQUENCY QUESTIONNAIRE

This questionnaire determines your usual eating habits and foods high in calcium

Record how often you eat each food (daily, weekly or monthly) and write the number in the corresponding column.

		How Often Do You Eat or Drink This Food or Beverage? (Fq)				
Food Source Containing Calcium		**Calcium Content mg**	**# of Times / Day**	**# of Time/ Week**	**# of Times/ Month**	**TOTAL**
Milk						
Milk	1 cup	240				
Milkshake	1.25 cup	360				
Sheep milk	1 cup	380				
Coco milk	1 cup	54				
Soy drink (enriched)	1 cup	240				
Soy drink	1 cup	26				
Rice drink	1 cup	22				
Oat milk	1 cup	16				
Almond milk	1 cup	90				
Yoghurt						
Yoghurt, flavored	5 oz.	197				
Yoghurt with fruit pieces	5 oz.	169				
Yoghurt natural	5 oz.	207				
Cheese						
Hard cheese (e.g. Cheddar, Gruyere, Emmental, Parmesan)	1 oz.	240				
Soft cheese (e.g. Camembert, Brie)	2 oz.	240				
Feta	2 oz.	270				
Mozzarella	2 oz.	242				
Fresh cheese (e.g. cottage cheese, ricotta, mascarpone)	7 oz.	138				
Cream cheese	1 oz.	180				
Cream. Desserts						
Cream, double, whipped	2 Tsp	21				
Cream, full	2 Tsp	21				

Custard made with milk, vanilla	4 oz.	111				
Ice cream, vanilla	3.5 oz.	124				
Pudding, vanilla	4 oz.	120				
Rice pudding	7 oz.	210				
Pancake	3 oz.	62				
Cheese cake	7 oz.	130				
Waffle	3 oz.	47				
Meat, Fish, and Egg.						
Egg	2 oz.	27				
Red meat	4 oz.	7				
Chicken	4 oz.	17				
Fish (e.g. Cod, Trout, Herring, Whitebait)	4 oz.	20				
Tuna (canned)	4 oz.	34				
Sardines in oil (canned)	2 oz.	240				
Smoked salmon	2 oz.	9				
Shrimp	5 oz.	45				
Beans and Lentils						
Lentils	1/3 cup raw 1 cup cooked	40				
Chick peas	1/3 cup raw 1 cup cooked	99				
White beans	1/3 cup raw 1 cup cooked	132				
Red beans	1/3 cup raw 1 cup cooked	93				
Starchy Foods						
Pasta (cooked)	¾ cup	26				
Rice (boiled)	¾ cup	4				
Potatoes (boiled)	1 cup	14				

White bread	1 slice	6				
Wholemeal bread (1 slice)	1 slice	12				
Muesli (cereals)	¼ cup	21				
Naan	2 oz.	48				
Fruits						
Orange	5 oz.	60				
Apple	4 oz.	6				
Banana	5 oz.	12				
Apricot (3 pieces)	4 oz.	19				
Currant (dried gooseberry)	4 oz.	72				
Figs, dried	2 oz.	96				
Raisins (dried grapes)	1.5 oz.	31				
Vegetables						
Lettuce	2 oz.	19				
Kale, Collard greens (raw)	2 oz.	32				
Bok Choy/Pak Choi (raw)	2 oz.	20				
Broccoli (raw)	4 oz.	112				
Gombo/Okra (raw)	4 oz.	77				
Cress (raw)	4 oz.	188				
Rhubarb (raw)	4 oz.	103				
Carrots (raw)	4 oz.	36				
Tomatoes (raw)	4 oz.	11				
Nuts and Seeds						
Almonds	1 oz.	75				
Walnuts	1 oz.	28				
Hazelnuts	1 oz.	56				
Brazil nuts	1 oz.	53				
Sesame seeds	1 Tbsp	22				
Tahini paste	1 oz.	42				
Processed Foods						
Quiche (cheese, eggs)	7 oz.	212				

Omelette with cheese	4 oz.	235				
Pasta with cheese	12 oz.	445				
Pizza	11 oz.	378				
Lasagna	11 oz.	228				
Cheeseburger	7 oz.	183				
Others						
Tofu	4 oz.	126				
Seaweeds	3.5 oz.	70				
Wakame	3.5 oz.	150				
Supplements						
Calcium	500 mg	500				
Calcium supplements – other amount						

This information is taken from the calcium calculator which was established by International Osteoporosis Foundation (IOF) Retrieved October 13, 2016, from https://www.iofbonehealth.org/calcium-calculator

Appendix 8

Food Diary (please note exercise / medications section)

Meal	Food	Amount (Please be as specific as you can, e.g. 1.5 cups, 3 TBS, etc.) Also Include method of preparation (Baked/Fried) and Brand name
Breakfast		
Lunch		
Dinner		
Snack 1		
Snack 2		

Activity Level (self-assessed)

❒ Low ❒ Medium ❒ High

Type of Activity_____

Medications (other than usual medication / also list new medications not included on original medical history form, e.g. Tylenol Cold & Flu, Aleve,etc.)

_____

Other concerns: